Waters 2489 UV/Visible Detector

Operator's Guide

71500142102/Revision B

Waters

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We seriously consider every customer comment we receive. You can reach us at tech_comm@waters.com.

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Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, and radiological hazards. You must know the potentially hazardous effects of all substances you work with. Always follow Good Laboratory Practice, and consult your organization's safety representative for guidance.

When you develop methods, follow the "Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory," *American Journal of Medical Technology*, 44, 1, pages 30–37 (1978). This protocol addresses good operating procedures and the techniques necessary to validate system and method performance.

Safety advisories

Consult Appendix A for a comprehensive list of warning and caution advisories.

Operating this instrument

When operating this instrument, follow standard quality-control (QC) procedures and the guidelines presented in this section.

Applicable symbols

Symbol	Definition
	Confirms that a manufactured product complies with all applicable European Community directives
ABN 49 065 444 751	Australia C-Tick EMC Compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	This product has been tested to the requirements of CAN/CSA-C22.2 No. 61010-1, second edition, including Amendment 1, or a later version of the same standard incorporating the same level of testing requirements

Audience and purpose

This guide is intended for personnel who install, operate, and maintain 2489 UV/Visible detectors.

Intended use of the 2489 UV/Visible detector

Waters designed the 2489 UV/Visible detector to analyze and monitor many compounds.

Calibrating

To calibrate LC systems, follow acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for standards should include the entire range of QC samples, typical specimens, and atypical specimens.

When calibrating mass spectrometers, consult the calibration section of the operator's guide for the instrument you are calibrating. In cases where an overview and maintenance guide, not operator's guide, accompanies the instrument, consult the instrument's online Help system for calibration instructions.

Quality-control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. Ensure that QC sample results fall within an acceptable range, and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report these data until you are certain that the instrument performs satisfactorily.

ISM classification

ISM Classification: ISM Group 1 Class B

This classification has been assigned in accordance with CISPR 11 Industrial Scientific and Medical (ISM) instruments requirements. Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment. Class B products are suitable for use in both commercial and residential locations and can be directly connected to a low voltage, power-supply network.

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Table of Contents

Copyright notice ii

Trademarks ii

Customer comments iii

Contacting Waters iv

Safety considerations iv
- Safety advisories v

Operating this instrument v
- Applicable symbols v
- Audience and purpose v
- Intended use of the 2489 UV/Visible detector v
- Calibrating vi
- Quality-control vi

ISM classification vi
- ISM Classification: ISM Group 1 Class B vi

EC Authorized Representative vii

1 Theory and Principles of Operation 1-1

Detector description 1-2

Principles of operation 1-4
- Detector optics 1-4
- Wavelength verification and test 1-9

Operational modes 1-10
- Single-wavelength mode 1-10
- Dual-wavelength mode 1-11
- Spectrum scanning 1-12
- Cuvette operations 1-13
- RatioPlot 1-14
- MaxPlot 1-14

Thermal-wander management 1-14

2 Installing the Detector 2-1

Preparing for installation 2-2

Site selection and power requirements 2-3
Site selection 2-3
Power requirements 2-4
Unpacking and inspecting 2-5

Making plumbing connections 2-6

Making electrical power connections 2-9
Making signal connections 2-10
Making Ethernet connections 2-13

Connecting to other devices 2-19
Connecting the detector to Empower using an e-SAT/IN module 2-20
Connecting the detector to a 745/745B/746 data module 2-23
Connecting the detector to a chart recorder 2-25
Connecting the detector to the Waters 600 series pump 2-28
Connecting the detector to the Waters 717plus autosampler 2-31
Connecting the detector to a fraction collector 2-33

3 Preparing the Detector 3-1

Initializing the detector 3-2
Idle mode 3-3

Using the operator interface 3-4
Using the display 3-4
Using the keypad 3-7
Navigating the user interface 3-12
Primary and secondary functions 3-14
Operating other detector functions 3-22
Operating the detector 3-26
Verifying that the detector operates properly 3-27
Wavelength calibration 3-30
Operating the detector in single-wavelength mode 3-30

Operating the detector in dual-wavelength mode 3-31
Programming timed events, threshold events, and methods 3-34

Scanning spectra 3-42
Before you begin 3-42
Scanning new spectra 3-46
Storing a spectrum 3-53
Getting information about a stored spectrum 3-54
Reviewing a stored spectrum 3-55
Subtracting a spectrum 3-55
Replaying a spectrum 3-56
Scanning using the cuvette 3-56
Scanning using a flow cell and a syringe 3-59
Conserving lamp life 3-60
Shutting down the detector 3-62

4 Maintaining the Detector 4-1

Contacting Waters technical service 4-2

Maintenance considerations 4-2
Safety and handling 4-3
Spare parts 4-3

Proper operating procedures 4-4
Removing the front left panel cover 4-4
Routine maintenance 4-5

Maintaining the flow cell 4-5
Flushing the flow cell 4-6
Removing and cleaning the flow cell 4-7
Disassembling and reassembling the flow cell 4-8

Replacing the lamp 4-16
Removing the lamp 4-18
Installing the new lamp 4-21
Recording the new lamp serial number 4-23
Setting the lamp threshold 4-24

Replacing fuses 4-25

5 Error Messages, Diagnostic Tests, and Troubleshooting 5-1

Error messages 5-2
Startup error messages 5-2
Error messages preventing operation 5-5

User-selected diagnostic tests 5-9
Overview 5-9
Using the diagnostic tests 5-11
Service diagnostic tests 5-19

Troubleshooting 5-20
When you contact Waters 5-20
Diagnostic tests 5-20
Power surges 5-21
Hardware troubleshooting 5-21

A Safety Advisories A-1

Warning symbols A-2
Task-specific hazard warnings A-2
Specific warnings A-3

Caution symbol A-5

Warnings that apply to all Waters instruments A-5

Electrical and handling symbols A-12
Electrical symbols A-12
Handling symbols A-13

B Specifications B-1

Operational specifications B-2

Optical specifications B-4

Optional Waters TaperSlit flow cell specifications B-5

C Solvent Considerations C-1

Introduction C-2
Preventing contamination C-2
Clean solvents C-2
Solvent quality C-2
Preparation checklist C-2
Water C-3
Using buffers C-3
Tetrahydrofuran C-3

Solvent miscibility C-3
How to use miscibility numbers C-5

Buffered solvents C-6

Head height C-6

Solvent viscosity C-6

Mobile phase solvent degassing C-7
Gas solubility C-7

Solvent degassing methods C-8
Sparging C-8
Vacuum degassing C-8
Solvent degassing considerations C-9

Wavelength selection C-9
UV cutoffs for common solvents C-10
Mixed mobile phases C-10
Wavelength selection for chromophore detection C-11

1 Theory and Principles of Operation

This chapter summarizes the Waters® 2489 UV/Visible Detector features and describes the theory and principles of operation.

Contents:

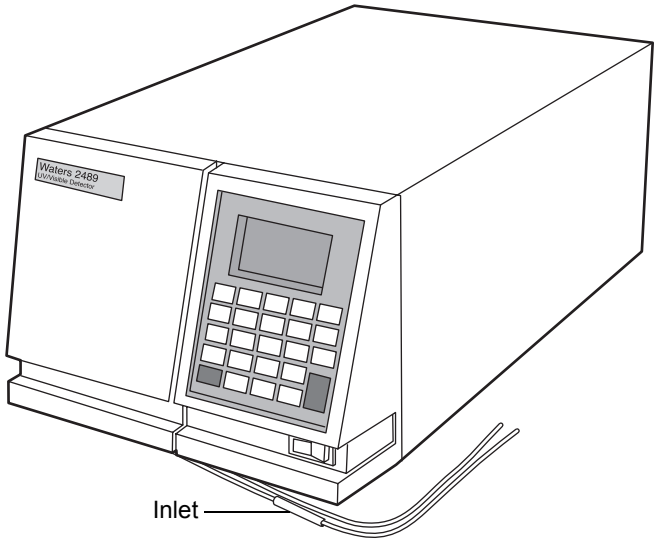
Topic	Page
Detector description	1-2
Principles of operation	1-4
Operational modes	1-10

See also: Appendix B for system specifications and Appendix C for information on high-performance liquid chromatography (HPLC) solvent considerations.

Detector description

The Waters 2489 UV/Visible Detector is a two-channel ultraviolet/visible (UV/Vis) detector designed for high-performance liquid chromatography (HPLC) applications.

Waters 2489 UV/Visible Detector



The detector can operate as a stand-alone unit (with a chart recorder or integrator) or as an integral part of a Waters chromatography system.

The detector can be configured with Empower™ or MassLynx™ software systems.

The detector has the following capabilities:

- Stand-alone programmability – Stores as many as five user-defined programs (or methods) consisting of as many as 50 programmable, timed events and two threshold events each.
- Single or dual-wavelength – Monitors absorbance at one or two discrete wavelengths.
- Wavelength verification reference filter – Ensures wavelength accuracy.
- Automatic, second-order filter – Automatically engaged for wavelengths of 370 nm and greater and removed for wavelengths 369 nm or less.
- Spectrum scan and storage – Supports spectrum scan, display, subtraction, storage, and playback, in addition to standard absorbance and UV/Vis functionality.
- Cuvette qualification – Facilitates qualification of the detector by insertion of a standard in a cuvette without breaking any plumbing connections. Waters qualification kits, available in cuvette form, support this feature, which allows the detector to serve as a benchtop spectrophotometer.
- Cuvette sample analysis – Allows recording of the spectrum of any sample placed in the cuvette.
- Method editing and storage – Supports basic method programming, storage, and retrieval from the front panel.
- Full diagnostic capability – Supports built-in diagnostic tools to optimize functionality and performance.
- Two contact closure outputs – The detector has two configurable switches, each of which can accommodate a maximum of ±30 VDC, 1.2-A current carrying capacity, and 0.5-A current switching. The switches (SW1 and SW2) can trigger fraction collectors and other external devices, as well as activate according to time, absorbance threshold, or ratio criteria.
- Therma- wander management – To mitigate thermal instability caused by ambient temperature changes, the detector's insulation ensure air flow across the optics bench, and a variable speed fan that runs at higher or lower speeds, as needed.
- Median Baseline Filter (MBF) – A variation of the data mode, the MBF decreases the effects of gradient separations on the chromatographic baseline. It enhances the UV detector's baseline stability by decreasing its curvature, making the development of integration methods easier.

Principles of operation

To use the detector effectively, become familiar with its optical and electronic design and the theory and principles of its operation.

This section describes the following parts and functions of the detector:

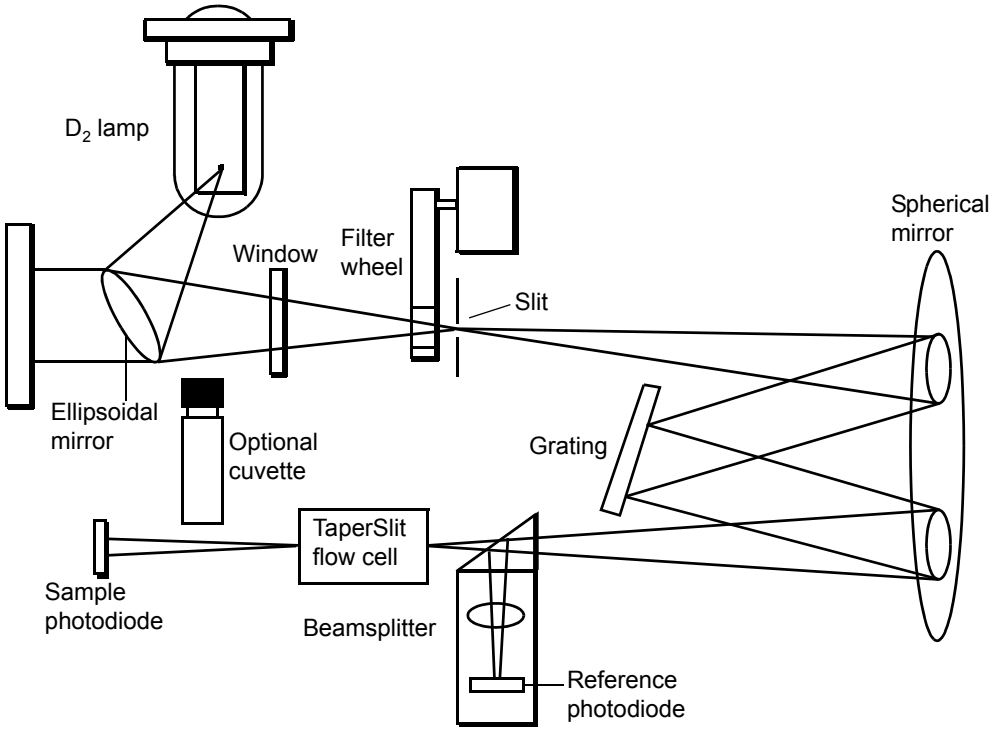
- Optics
- Wavelength verification and test
- Flow cell
- Electronics

Detector optics

The Waters 2489 UV/Visible detector optics are based on a Fastie-Ebert monochromator and include these components:

- High brightness deuterium (D_2) lamp
- Two mirrors: one, off-axis, ellipsoidal mirror and one spherical mirror
- Filter wheel
- Shutter, wavelength calibration filter, and second-order filter
- Entrance slit
- Blazed, plane-holographic, diffraction grating
- Beamsplitter
- Sample and reference photodiodes
- Waters TaperSlit™ Flow Cell (its entrance is the exit slit of the monochromator)
- Cuvette holder

Waters 2489 UV/Visible detector optics assembly



Optics assembly light path

The detector provides an extremely efficient design for exceptionally high light throughput. It operates as follows:

1. The ellipsoidal mirror collects light from the lamp and focuses it through the filter wheel onto the entrance slit. The spherical mirror directs light toward the grating. A different portion of the spherical mirror focuses dispersed light of a particular wavelength band, determined by the grating angle, onto the entrance of the flow cell. Light exiting the flow cell passes through the cuvette location to the sample photodiode.
2. The beamsplitter, located just ahead of the flow cell, diverts a portion of the light to a reference photodiode.
3. When you enter a new wavelength through the detector's front panel (or through Empower or MassLynx software), the detector rotates the grating to the appropriate position.

4. The preamplifier board integrates and digitizes the currents from the photodiodes for processing by the signal processing electronics and output to a computer, chart recorder, or integrator.

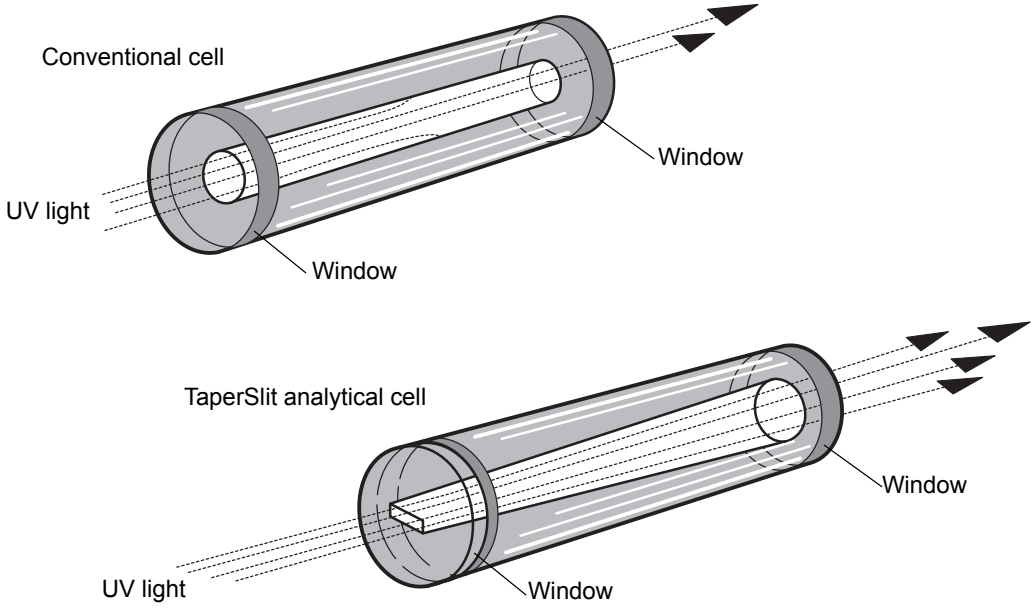
Waters TaperSlit Flow Cell

The Waters TaperSlit Flow Cell used in this detector renders the detector baseline less sensitive to changes in mobile phase refractive index (RI). RI changes occur during gradient separations or result from temperature or pump-induced pressure fluctuations.

To achieve RI immunity, a combination of a spherical mirror, a lens at the entrance of the flow cell, and a taper to the internal bore of the flow cell prevents light rays from striking the internal walls of the flow cell. An additional feature of the TaperSlit flow cell and the reason for its name is the shape of the flow cell entrance, which matches the shape of the entrance slit. The detector achieves higher light throughput for a given spectral resolution via the TaperSlit cell design, compared to a conventional flow cell with a circular entrance.

As shown in the figure below, in a conventional cell, light bends and hits the wall of the flow cell. Four beams go in, but only two come out. In the Waters TaperSlit analytical cell, the combination of the lens and TaperSlit bore geometry prevents light from hitting the cell walls. Four beams go in, and four beams come out.

Comparison of flow cell characteristics



The standard analytical, inert, and LC/MS cells have a path length of 10 mm. The semi-prep and microbore cell path length is 3 mm. The autopurification cell path length is 1.0 mm. A variable path length flow cell (path length 0.15 to 3 mm) is also available.

Filtering noise

The detector provides a Hamming filter to minimize noise. The Hamming filter is a digital finite impulse response filter, which creates peak height degradation and enhances the filtering of high frequency noise.

The behavior of the filter depends on the filter time-constant you select. You can program a filter time to be Fast, Slow, Normal, or Other. If you select Fast, Slow, or Normal, you do not need to enter a value. The filter constant is determined by the data rate. If you select Other, you can enter a value. However, the value you enter is rounded up or down to a value based on the data rate.

The filter time-constant adjusts the filter response time to achieve an optimal signal-to-noise ratio. Selecting Other and entering a value of 0.0 disables all filtering.

Lower time-constant settings produce these effects:

- Narrow peaks with minimal peak distortion and time delay
- Very small peaks become harder to discriminate from baseline noise
- Less baseline noise is removed

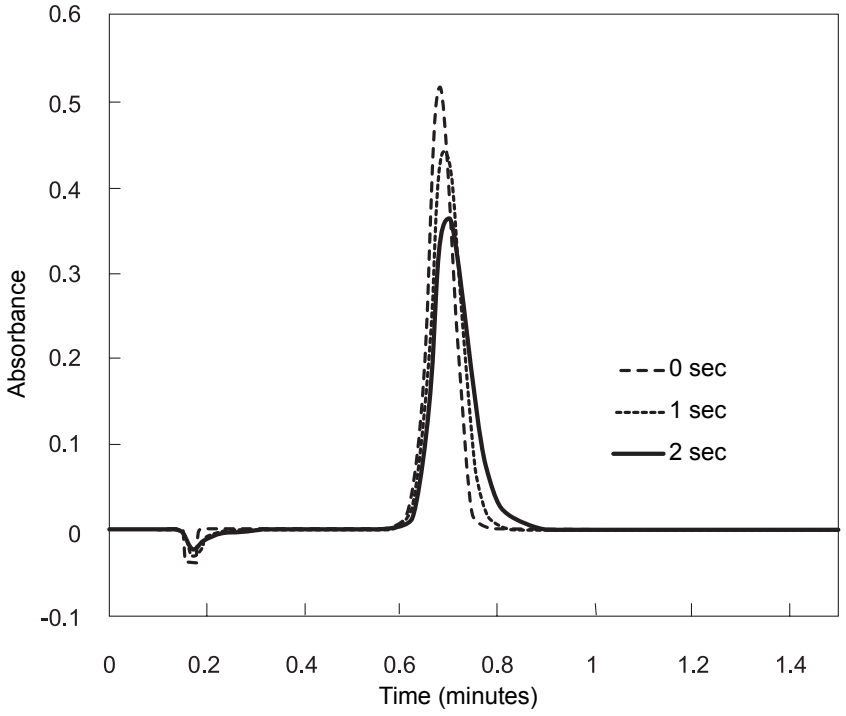
Higher time-constant settings produce these effects:

- Greatly decrease baseline noise
- Shorten and broaden peaks

The software includes fast or normal filtering constants at each data rate that are appropriate for high speed or high sensitivity applications respectively.

The following figure shows the relationship between increased filter time-constant and absorbance.

Filter time-constant comparison



Tip: Although the peak shape shows some distortion and the signal output is delayed with different time-constants, the peak area remains the same.

Wavelength verification and test

The detector's deuterium arc lamp and integral erbium filter exhibit peaks in the transmission spectrum at known wavelengths. Upon startup, the detector verifies calibration by comparing the locations of these peaks with expected wavelengths based on calibration data stored in the detector's memory. If the results of this verification differ from the stored calibration by more than 1.0 nm, the detector displays a Wavelength Verification Failure message. The detector verifies, rather than recalibrates, on startup to avoid errors arising from residual materials left in the flow cell and/or the cuvette.

Requirement: Always ensure that the cuvette is removed and the cuvette holder and front, left-hand panel door are secured during startup verification.

You can initiate a manual wavelength calibration at any time. A manual calibration replaces the previous calibration data with new data. See "Wavelength calibration" on page 3-30 for the manual wavelength calibration procedure.

The verification and calibration algorithms are virtually identical. However, the verification algorithm can issue an error message indicating that actual data do not match stored data where the calibration algorithm replaces the stored data with the new.

The detector wavelength verification procedures establish an approximate Home position using a grating homing sensor. Once Home is established, the detector locates and references the 656.1-nm peak in the deuterium lamp emission spectrum.

The integral erbium filter moves into the common light path ahead of the flow cell entrance slit, enabling the detector to locate three additional spectral features at these wavelengths:

- 256.7 nm (UV)
- 379.0 nm
- 521.5 nm

The verification tests for the detector require five minutes of lamp warmup time.

If you run the detector continuously, Waters recommends that you perform wavelength verification weekly by turning off the detector, then turning it on again. See "Wavelength calibration" on page 3-30.

Operational modes

The detector operates in single or dual-wavelength mode, allows spectrum scanning using a flow cell or a cuvette, and provides RatioPlot, difference plot, and MaxPlot functions.

Single-wavelength mode

Single-wavelength is the default mode of operation for the detector. The detector supports monitoring of a single-wavelength from 190 nm to 700 nm, settable in 1-nm increments on channel A. You can configure the analog outputs for channel B while the detector is operating in single-wavelength mode, so you can use channel B to obtain additional information about the wavelength selected on channel A.

In single-wavelength mode, the detector automatically engages the second-order filter for wavelengths 370 nm and above and removes it for wavelengths under of 370 nm. The second-order filter is an optical filter that blocks unwanted ultraviolet (UV) light from striking the diffraction grating and interfering with absorbance detection above 370 nm.

You can configure several additional parameters when using the detector in single-wavelength mode.

Primary parameters

The following are the values of major parameters that apply to single-wavelength mode:

- Wavelength, in nanometers – Specifies a wavelength for channel A, from 190 nm to 700 nm, settable in 1-nm increments.
- Sensitivity in AUFS – Specifies the scaling factor for the analog output channels and corresponds to the absorbance unit (AU) value where the analog outputs saturate at full-scale values. Absorbance units full-scale (AUFS) vary from 0.0001 to 4.000 AU.

  Tip: Changing the sensitivity (AUFS) setting affects the 2-V output.
- Chart polarity (+ or –) – Reverses the polarity of the charted chromatogram. Select + for a normal chromatogram, or – for an inverted chromatogram. This function changes the direction of the plot on the 2-V output, similar to reversing the leads to an external chart recorder.

- Filter time-constant – Programs a filter time in seconds. Options are Fast, Slow, Normal, or Other. If you select Fast, Slow, or Normal, you do not need to enter a value. The filter constant is determined by the data rate. If you select Other, you can enter a value, but the value you enter is rounded up or down to a value based on the data rate. Selecting Other and entering a value of 0.0 disables all filtering.
- Analog rate – Specifies a value as many as 80 Hz.

Secondary parameters

Pressing Next on the absorbance (or HOME) screen while in single-wavelength mode, causes several pages of these secondary, or less frequently specified, parameters to appear:

- Absorbance offset (in mV)
- Auto zero on inject
- Auto zero on λ changes

"Primary and secondary functions" on page 3-14 and the table on page 3-5 explain the functions, ranges, and defaults of these parameters.

Dual-wavelength mode

In dual-wavelength mode, the detector can monitor two wavelengths, one on channel A and one on channel B. The sampling frequency is reduced to 1 or 2 Hz, limiting use of this mode to more standard chromatography where peaks span at least 20 seconds to enable full characterization of a peak. You can use dual-wavelength mode to obtain additional information about an analyte by running in the RatioPlot or MaxPlot mode.

You select any two wavelengths from 190 nm to 700 nm.

In dual-wavelength mode, the following conditions apply:

- If both selected wavelengths are greater than 370 nm, the detector applies the second-order filter to block unwanted UV light.
- If both selected wavelengths are less than or equal to 370 nm, the detector removes the second-order filter.
- If the selected wavelengths bracket the 370 nm threshold, the detector does not apply the second-order filter and issues a warning message that any data collected for the wavelength above 370 nm can contain

inaccuracies because of possible UV light interference (second-order effects).

Chart-out selection modes

When operating in dual-wavelength mode, the detector offers these choices for analog output in addition to the selections offered in single-wavelength mode and explained on page 1-10. The default selection for dual-wavelength mode is Absorbance.

- Absorbance (A and B) – The standard LC mode where the current absorption is scaled and sent directly out the analog output. The scaling depends on the AUFS setting and the absorbance offset. The absorbance value is scaled for the 2-V analog output. If a setting of 1 AU/V is desired, you can set an AUFS of 2.0000 for either the A or B output channels that can be controlled independently, even in single-wavelength mode.
- MaxPlot – This mode results in the output of the larger of the two absorbance values scaled to the selected AUFS setting. Use this mode when observing, with one data channel, multiple compounds that exhibit absorbancies at two separate wavelengths.
- RatioPlot (A/B) – This mode produces the ratio of absorbance from two wavelengths. Theoretically, the ratio is constant for a pure chromatographic peak and variable for an impure peak, which results in a nonsquared response. Instead of a programmable AUFS, the detector provides minimum and maximum ratio values that scale the ratio plot proportionally. In addition, a configurable minimum absorbance threshold activates ratio output scaling only when it reaches the absorbance at both wavelengths.
- Difference Plot (A-B) – This mode plots the arithmetic difference in absorbance for the two monitored wavelengths.

Spectrum scanning

When the detector is operating under the control of the Empower software, the scanning function is disabled.

You can use the detector as a spectrophotometer, to acquire spectra from either the flow cell or the cuvette. You can scan and store as many as three spectra (three reference or zero scans, or three sample scans) for playback or to compare with other spectra.

The major difference between the detector and a double-beam spectrophotometer is that the detector uses only one flow cell or cuvette, rather than a sample and a reference pair.

Recommendation: Use a matched pair of cuvettes for the zero and sample scans.

The detector obtains an absorbance spectrum by performing two types of scans on the flow cell or using the cuvette:

- Zero scan – Characterizes the baseline absorbance spectrum of a solvent.
- Sample scan – Subtracts the zero scan, so the results displayed or charted are of the sample only.

To obtain a spectrum of a sample using the detector, run a zero scan first, followed by a sample scan. Typically, you run the zero scan using pure solvent. The sample scan is a scan of the analyte dissolved in that solvent.

Spectra can be simultaneously charted on the channel A output, or acquired and stored in memory for later playback.

See also: "Scanning using the cuvette" on page 3-56 and "Scanning using a flow cell and a syringe" on page 3-59.

Cuvette operations

The detector cuvette option is used to measure the absorbance spectrum of a sample in a cuvette.

To generate and store a spectrum

1. Acquire a zero scan, which measures the absorbance of the contents of the cuvette and flow cell over the desired wavelength range.
2. Acquire a sample (absorbance) scan, which measures the absorbance of the analyte dissolved in mobile phase.

The detector subtracts the zero scan from the sample scan to create a sample spectrum.

Since the cuvette scan is acquired by measuring the absorbance from a light path that includes both the flow cell and the cuvette, the solvent conditions in the flow cell must be identical for both scans. (See "Scanning using the cuvette" on page 3-56.)

RatioPlot

The detector allows ratio plotting: comparing the absorbances of a compound or analyte at two wavelengths. The RatioPlot divides absorbances at two selected wavelengths and plots the resulting ratio on a chart recorder or data system over one output channel (channel A). Use the RatioPlot when detecting hidden components within individual peaks.

The RatioPlot of a spectrally homogeneous peak appears as a rectangular wave. The RatioPlot of an impure peak appears as a distorted wave. When obtaining a RatioPlot, you must be operating the detector in dual-wavelength mode; the RatioPlot is output on the selected channel. (See page 3-33.)

MaxPlot

The MaxPlot function monitors absorbance at two selected wavelengths and plots the maximum absorbance value for each sample component. To obtain a MaxPlot, you must operate the detector in dual-wavelength mode. The MaxPlot outputs the greater of the two absorbance values on the selected channel.

For an explanation of the MaxPlot procedure, see the discussion "Obtaining a MaxPlot" on page 3-34.

Thermal-wander management

To mitigate thermal instability caused by ambient temperature changes, the the detector's variable speed fan runs at higher or lower speeds, as needed. The fan normally changes speeds in response to the thermal changes.

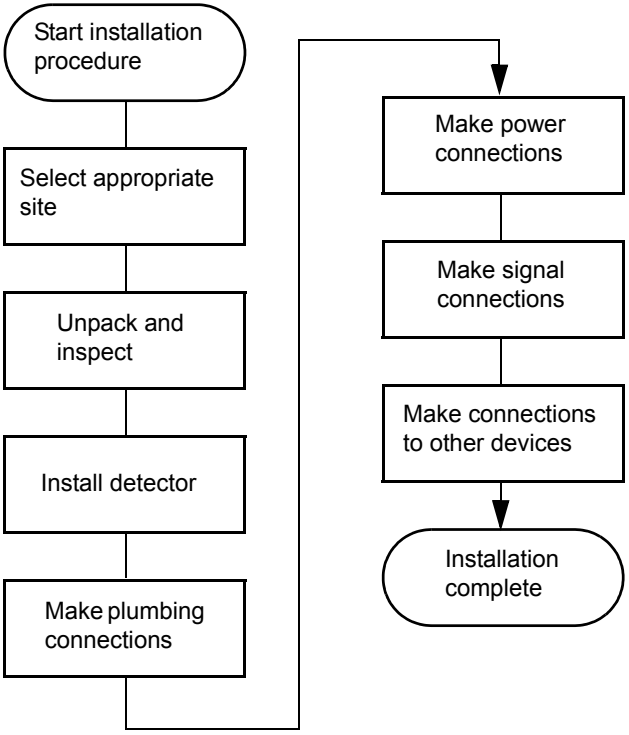
2 Installing the Detector

The detector requires connections to electrical power and to sample and waste lines to operate in any standard laboratory environment. This chapter describes how to install the detector, and connect it to the electrical supplies and to other equipment in an HPLC system.

Contents:

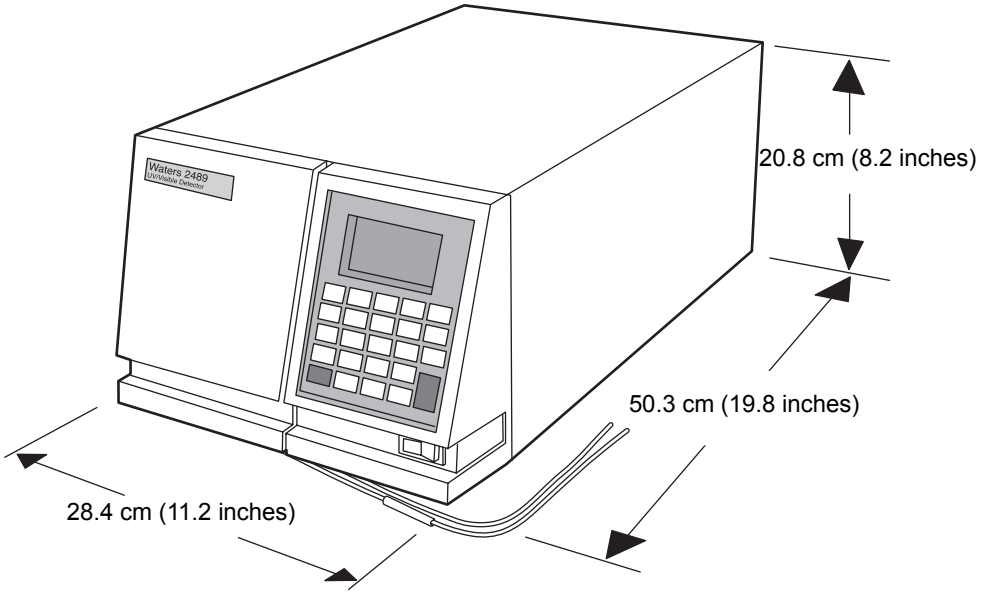
Topic	Page
Preparing for installation	2-2
Site selection and power requirements	2-3
Making plumbing connections	2-6
Making electrical power connections	2-9
Connecting to other devices	2-19

Major steps in installing the detector



After installing the detector, verify its function, and keep the verified chart output (if applicable) on file. Verification ensures proper operation of the detector optics and electronics. For proper verification procedures, see "Verifying that the detector operates properly" on page 3-27.

Detector dimensions



Caution: Do not access the instrument through the top cover but through the front, left-hand panel, where the lamp housing, flow cell assembly, and cuvette holder are located.

Site selection and power requirements

Site selection

Install the detector in an area that meets the requirements listed in the table below.

Installation site requirements

Parameter	Requirement
Operating temperature range	4 to 40 °C (39 to 104 °F)

Installation site requirements (Continued)

Parameter	Requirement
Storage temperature range	-30 to 60 °C (-22 to 140 °F)
Relative humidity	20% to <95%, noncondensing
Storage humidity range	0 to <95%, noncondensing
Bench space	12.7 cm (5 inches) clearance at rear
Vibration	Negligible
Static electricity	Negligible
Power	Grounded AC, 100/240 VAC, 50/60 Hz Power cord type required: • SVT, in the United States • HAR type (or better), in Europe For information regarding the type of cord to use in other countries, contact your local Waters distributor.

Requirement: You must mount the detector on a level surface to allow proper function of the drip management system (drain tube), which you can connect to a waste reservoir to divert solvent leaks from the flow cell.

Power requirements

Warning: To avoid electric shock,

- use the SVT-type power cord in the United States and HAR-type (or better) cord in Europe.
- power-off and unplug the detector before performing any maintenance procedures on the instrument.
- connect all HPLC system components to a common ground.

The detector requires

- grounded alternating current (AC) power source.
- minimal power transients and fluctuations.
- a line voltage of 100 to 240 VAC. (Power consumption is 185 VA.)

- operation in nominal voltage range of 100 to 240 VAC.
- two 100 to 240-VAC, 50 to 60-Hz, F 3.15-A, 250-V fast blo, 5 × 20 mm (IEC) fuses

Warning: For continued protection against fire hazard, replace fuses only with those of the same type and rating.

Unpacking and inspecting

The detector is packed and shipped in one carton, which contains the following items:

- Waters 2489 UV/Visible detector startup kit, which includes this guide
- Power cord
- Release notes

Unpacking

To unpack the detector

1. Unpack the contents of the shipping carton. As you unpack the carton, check the contents to make sure you have received all items.
2. Check the contents of the startup kit.
3. Save the shipping carton for future transport or shipment.

Inspecting

If you discover any damage or discrepancy when you inspect the contents of the cartons, immediately contact the shipping agent and your local Waters representative.

Customers in the USA and Canada can report damage and discrepancies to Waters Technical Service (800 252-4752). Others can phone their local Waters subsidiary or Waters corporate headquarters in Milford, Massachusetts (USA), or visit www.waters.com.

For complete information on reporting shipping damages and submitting claims, see *Waters Licenses, Warranties, and Support Services*.

Tip: Make sure the instrument serial number, found on the nameplate on the rear panel or inside the front left panel of the detector, corresponds to the number on the instrument integrity certificate.

Making plumbing connections

Before initially starting the detector

- Complete the plumbing connections described in this section.
- Complete the electrical connections (see "Making electrical power connections" on page 2-9).

Caution: Observe Good Laboratory Practices when handling solvents. Refer to the Material Safety Data Sheets for the solvents in use.

Requirement: You must make the following plumbing connections on your detector unit:

- Column connections
- Drip management system connections

Recommendations:

- Before completing the column connections, perform the verification procedures described in "Verifying that the detector operates properly" on page 3-27.
- Install a waste reservoir connected to the drain tube and located next to the rubber foot on the front, lower, left-hand section of the detector.
- Use Tygon® tubing to connect the drain tube to the waste reservoir.

Caution: The detector, as shipped, has a standard analytical flow cell, pressure-rated at 6895 kPa (69 bar, 1000 psi). To prevent damage, do not connect any tubing or device that can cause backpressure to exceed the pressure rating of the tubing or flow cell.

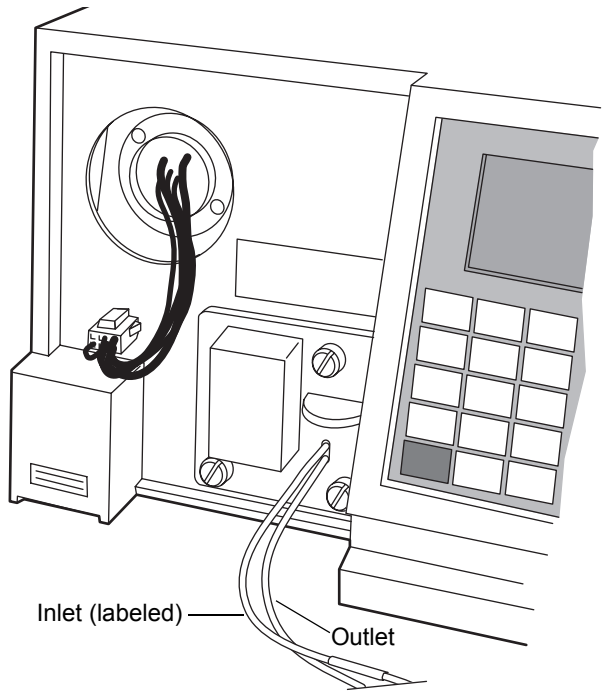
Connecting columns

The line connections to the detector are located in the front, right-hand side of the flow cell assembly (see the figure below).

To make the inlet and outlet tubing connections

1. Attach the stainless steel compression fitting and ferrule (supplied in the startup kit).
2. Connect the inlet tubing to the column outlet. Ensure the tubing is seated firmly, and then tighten the compression screw.
3. Connect the Tygon tubing to the flow cell outlet tubing, and route the tubing to a waste container.

Plumbing connections

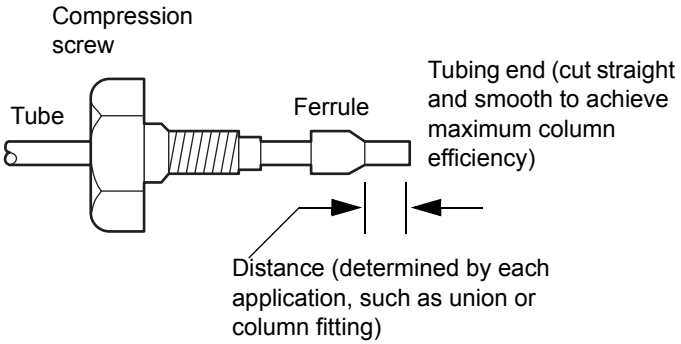


Assembling the fittings

To assemble each fitting

1. Slide the compression screw over the tubing end, followed by the ferrule.
2. Mount the ferrule with its tapered end facing the end of the tubing.

Ferrule and compression screw assembly



Making connections

To make connections at the column outlet and detector inlet, and at the detector outlet

1. Seat each tubing end in the appropriate fitting.
2. Seat each ferrule by tightening the compression screw 1/2-turn past finger-tight.

Requirement: To ensure accurate verification, power-on the detector before pumping any mobile phase or solvent through the flow cell.

Recommendation: To prevent reabsorption of dissolved oxygen (for systems using vacuum degassers), run the solvent degasser continuously when operating the detector at wavelengths less than 230 nm.

Making electrical power connections

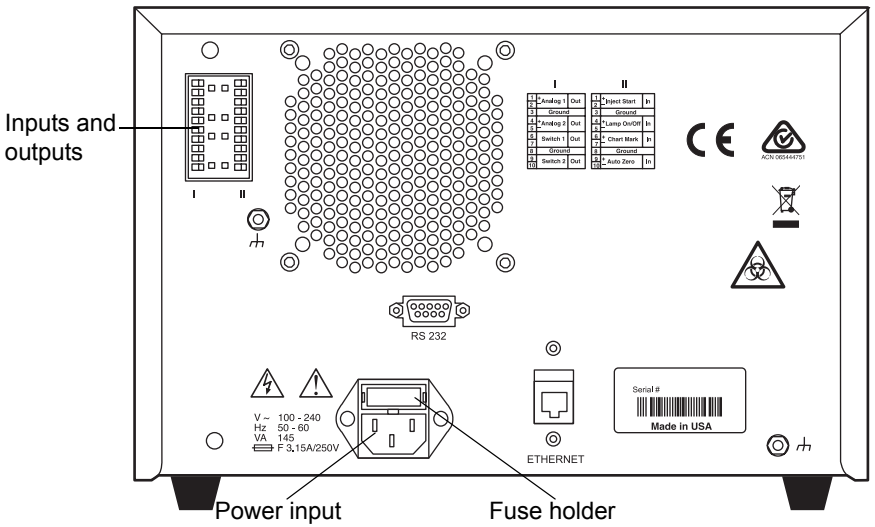
To connect the detector to the AC power supply

1. Plug the receptacle end of the power cord into the AC input connector on the rear panel of the detector (see the figure below).
2. Plug the other end of the power cord into a properly grounded AC power source.

Detector rear panel

The detector connects to other Waters components through rear panel electrical connections.

Detector rear panel electrical connections



Rear panel connections enable the following signals:

- Analog outputs – There are two pairs of attenuated analog channel outputs. Each pair supports 2-V output to external devices or data systems. They are labeled I and II. For input/output voltage current specifications, see page B-2.
  - The 2-V output for I and II is scaled based on the AUFS (absorbance units full scale) setting for each channel. The detector does not

provide a traditional “unattenuated”, dedicated, 1-V/AU output because its enhanced working range is above 2 AU.

  - The analog output range specifications are -0.1 V to 2.1 V.
  - You can set the AUFS value individually for the output on each channel. Volts per AU are calculated as follows:

    Volts out = Absorbance × 2V/AUFS

    Example: An AUFS setting of 2.0000 provides a traditional 1-V/AU output. An AUFS setting of 4.0000 provides a 0.5-V/AU output, which supports chromatography above 2 AU.

- Switched outputs – You can program two switch contact closures to turn on; turn off; toggle; pulse once, for a defined duration; or pulse repetitively, for a specified period of time.
- Event inputs – Four general-purpose TTL contact closures on the detector A (inputs) terminal support the following functions:
  - Remote or inject start
  - Lamp on/off
  - Chart-mark input
  - Auto-zero
- Ethernet interface – The Ethernet connection on the rear panel of the detector provides remote control operation and direct data acquisition from Waters Empower and MassLynx workstations.

Making signal connections

The rear panel of the detector (see the figure on page 2-9) provides two analog connectors and an Ethernet communications port for operating the detector with external devices.

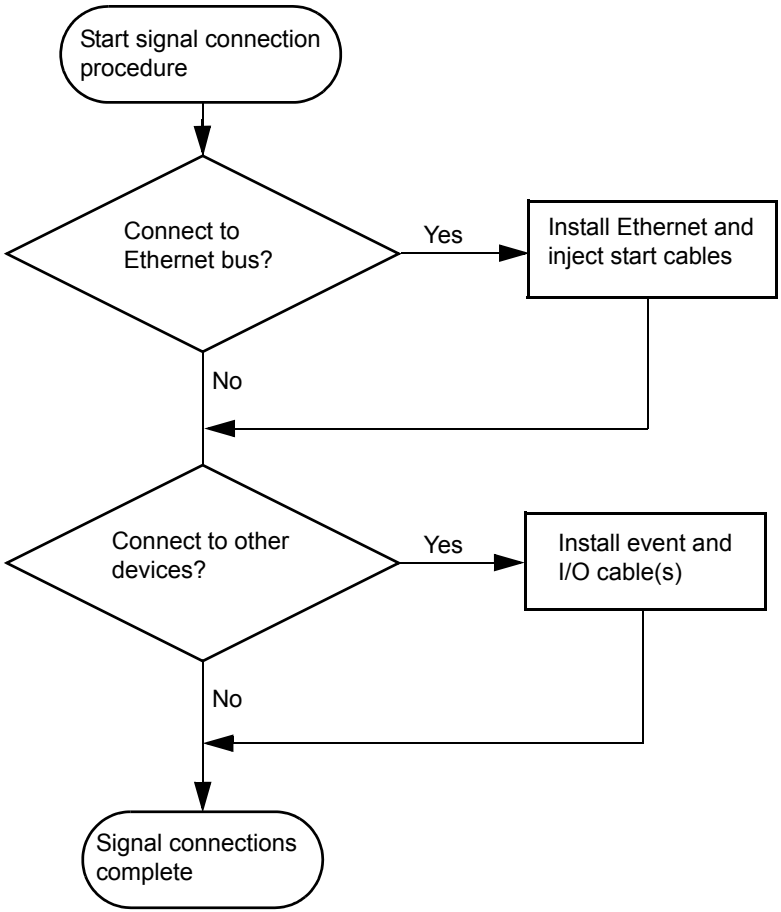
Make the signal connections to your detector, considering the following conditions:

- Operating mode you select for the detector (stand-alone or remote control)
- Types of instruments that make up your HPLC system

This section describes the input/output (I/O) and digital signal connections that you can make from the two rear panel connectors and the Ethernet connector.

The flow chart below provides an overview of the steps involved in making signal connections to the detector.

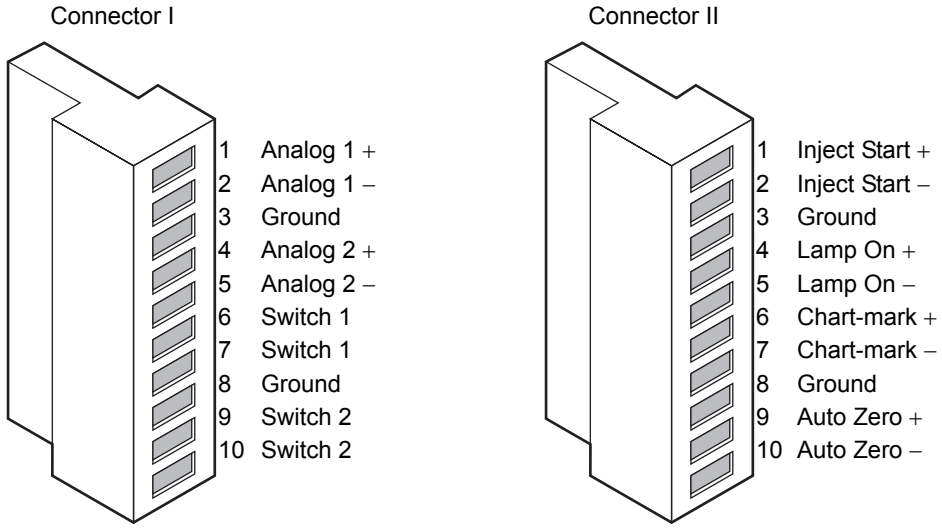
Overview of making signal connections



Making I/O signal connections

The rear panel includes two removable connectors that hold the pins for the I/O signals, as shown in the figure below. These connectors are keyed so that you can insert them one way only.

I/O signal inputs and outputs



I/O signals

The table below describes each of the signals available on the I/O connectors. See Appendix B for details on the electrical specifications for the signals.

I/O signals for the detector

Signal	Description
Inject Start[a]	TTL contact closure. Configurable input to initiate sequencing of time-programmed events. Defines the start of a run (typically an injection) and resets and starts the run-time clock to 0.00 minutes. Initial conditions apply immediately.
Lamp On/Off[a]	Configurable input to allow an external device to turn the deuterium lamp off and on.
Chart-mark[a]	Configurable input to add a chart-mark (at 10% of full scale) to either or both analog output channels.

I/O signals for the detector (Continued)

Signal	Description
Auto Zero[a]	Configurable input to auto-zero either or both channels.
Analog 1[b]	2-V full-scale analog output signal of channel A (scaled to the current AUFS setting).
Analog 2[b]	2-V full-scale analog output signal of channel B (scaled to the current AUFS setting).
Switch 1 (2)	Used to connect to a fraction collector. Can be controlled by threshold and timed events.
Switch 2 (2)	

a. Inject Start, Chart-mark, Auto-zero, and Lamp inputs are configurable from the detector first Configuration screen by setting the appropriate parameter to High. See "Configuring event inputs (contact closures)" on page 3-23 for more detail.

b. See the discussion of attenuation in the detector analog outputs in "Making electrical power connections" on page 2-9.

Making Ethernet connections

The detector's rear panel also includes one Ethernet interface connector for digital signal communications. Use this connector for devices such as these:

- Network adapter card in the Empower workstation
- Solvent manager
- MassLynx version 4.1 or higher

The Ethernet connector mates with standard Ethernet cable.

Caution: To avoid possible damage to components, power-off all instruments on the Ethernet connector before you connect an Ethernet cable to an instrument.

Making Ethernet connections with Waters data systems

When controlling the detector from a Waters data system or controller (Empower or MassLynx workstation), you can use the Ethernet interface to send and receive information from the data system.

When connecting via the Ethernet to these Waters data systems, you should be aware of the following:

- When in dual-wavelength mode, you must select a data rate of 1 point per second in the data system method editor. In Empower software, the data rate defaults to 1 point per second.
- The maximum range of the detector time-constant setting depends on the wavelength mode and data rate selected. See the page 3-18.
- Empower allows the detector to operate, in both single and dual-wavelength, modes with a wavelength range of 190 to 700 nm and as many as 4.0 AUFS.

To connect Ethernet cables from the detector to a Waters data system

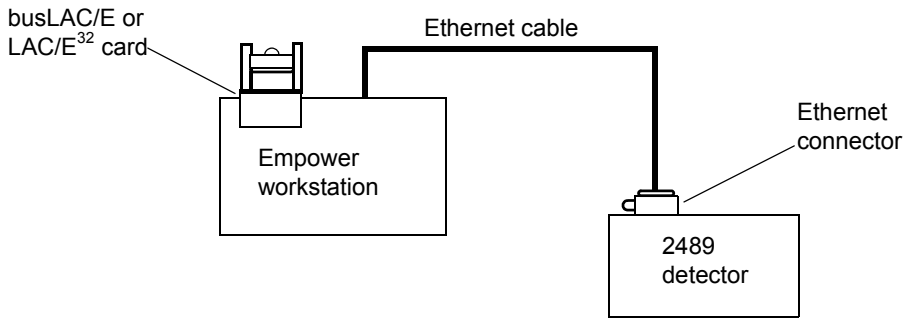
1. Connect the single receptacle end of the Ethernet cable to your data system by attaching the cable to the LAC/E interface.

   Note: The Ethernet cable is supplied with the Waters data system.

2. Connect the other end of the cable to the Ethernet connector on the rear panel of the detector.

   Caution: The maximum total cable length between Ethernet devices in a system is 20 meters (65 feet). The maximum recommended cable length between two Ethernet devices is 4 meters (13 feet). Longer total cable lengths can cause intermittent Ethernet communication failures.

Ethernet connections to the detector in a Waters Empower system



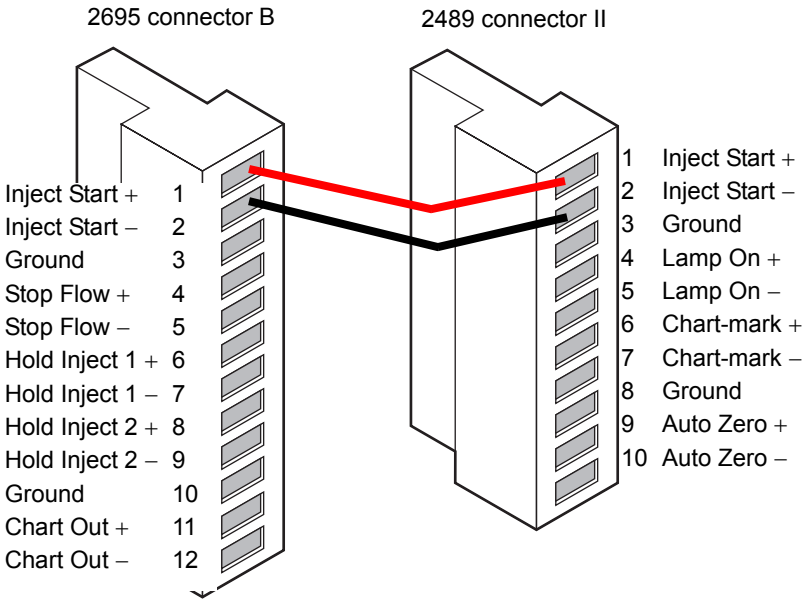
Tip: When connecting the detector to a data system, all detector parameters not configurable by the data system in use defer to local control.

Starting a method

To start a method on the detector at the start of an injection from the separations module, make the connections summarized in the table below and illustrated in the figure below that.

2695 separations module (B inputs and outputs)	2489 detector (II)
Pin 1 Inject Start	Pin 1 Inject Start +
Pin 2 Inject Start	Pin 2 Inject Start –

2695 separations module connections to the detector for starting a method



Note: If the injector is an e2695 separations module running in Ethernet mode or a 2707 autosampler, the inject start cable should not be connected. However, if the injector is an e2695 separations module running in IEEE mode, the inject start cable should be connected.

Turning the detector lamp on or off

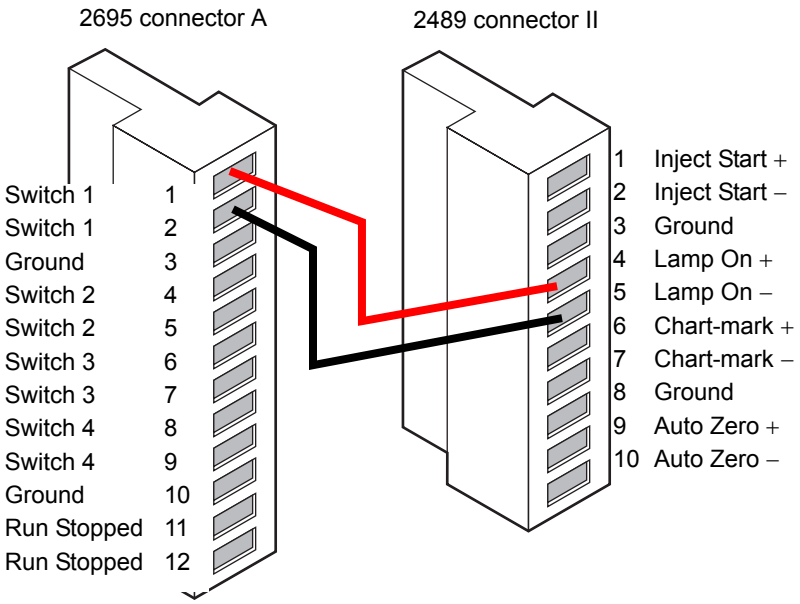
Before you can turn the detector lamp on or off from the separations module, you need to configure the lamp on/off signal at the front panel. You must change the default lamp configuration parameter setting from Ignore to High or Low. See the discussion "Configuring event inputs (contact closures)" on page 3-23 for more information.

After configuring the detector lamp on/off signal, you can turn the lamp on or off from the separations module by making the connections shown in the table and figure below.

Detector connections to separation module (lamp on or off)

2695 separations module (A outputs)	2489 detector (II)
Pin 1 Switch 1	Pin 4 Lamp On/Off +
Pin 2 Switch 1	Pin 5 Lamp On/Off –

Separations module connections to the detector for turning the lamp on or off



Connecting the detector to a separations module

You can connect the detector to the separations module when the detector is not under the control of the Empower software, to perform the following functions:

- Auto-Zero
- Chart-mark on Inject

Generating auto-zero

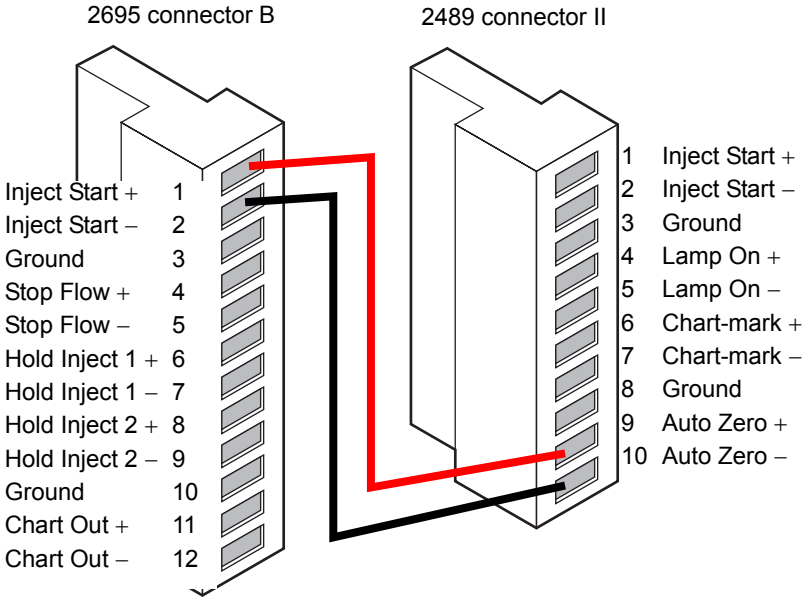
To generate the Auto-Zero function of the detector at the start of an injection, make the connections summarized in the table and illustrated in the figure the follows it.

Detector connections to a separation module to generate auto-zero

2695 separations module (B inputs and outputs)	2489 detector (II)
Pin 1 Inject Start	Pin 9 Auto Zero +
Pin 2 Inject Start	Pin 10 Auto Zero –

Before you can generate an auto-zero from the separations module, you need to configure the auto-zero signal at the detector's front panel. The default auto-zero signal is Low. See the discussion "Configuring event inputs (contact closures)" on page 3-23 for more information.

Separations module connections to the detector for auto-zero on inject



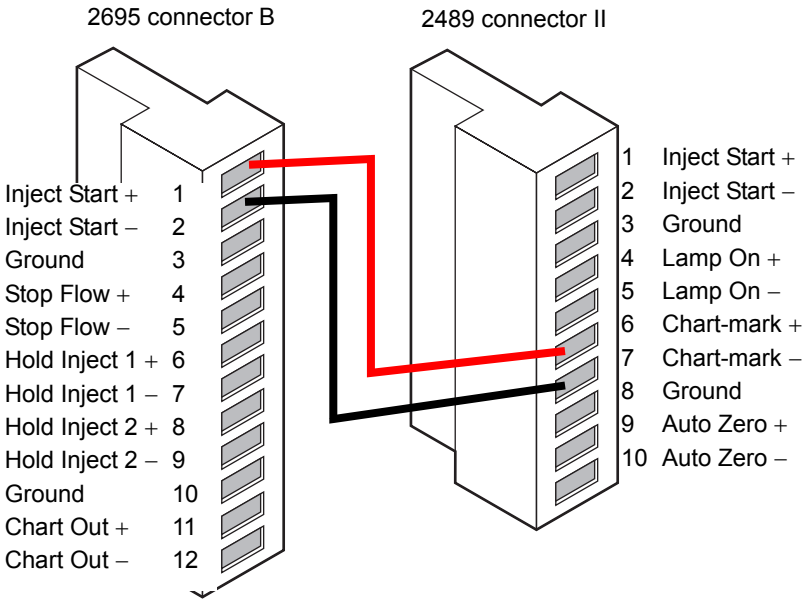
Generating a chart-mark on injection

To generate the chart-mark function on the detector at the start of an injection, make the connections summarized in the table and illustrated in the figure that follows it.

2695 separations module (B inputs and outputs)	2489 detector (II)
Pin 1 Inject Start	Pin 6 Chart-mark +
Pin 2 Inject Start	Pin 7 Chart-mark –

Before you can generate a chart-mark from the separations module, you need to configure the chart-mark signal at the detector's front panel. The default chart-mark signal setting is Low (see the discussion page 3-23 for more information).

Separations module connections to the detector for making a chart-mark on injection



Connecting to other devices

You can connect the detector to a many HPLC system devices. This section describes how to connect the detector to the following devices:

- e-SAT/IN™ module (instead of Ethernet)
- Waters 745/745B/746 Data Module
- Chart recorder
- Waters 600-series pump
- Waters 717plus autosampler
- Waters fraction collector

For details on connecting to other data modules, see the operator's guide for the module you are using.

Required materials

When connecting cables to the terminals on the rear panel of the detector, you need the following tools:

- Small, flat-blade screwdriver
- Electrical insulation stripping tool

Connecting signal cables

To connect cables from other HPLC system devices to the I and II terminals on the rear panel of the detector

1. Remove terminal I or II (see page 2-12).
2. Unscrew the connecting pin terminal.
3. Using the stripping tool, strip the wire about 3 mm (1/8 inch) from the end.
4. Insert the stripped wire into the appropriate connector.
5. Tighten the screw until the wire is held securely in place.
6. Reinsert the terminal.
7. Press firmly to ensure that it is inserted fully.

Connecting the detector to Empower using an e-SAT/IN module

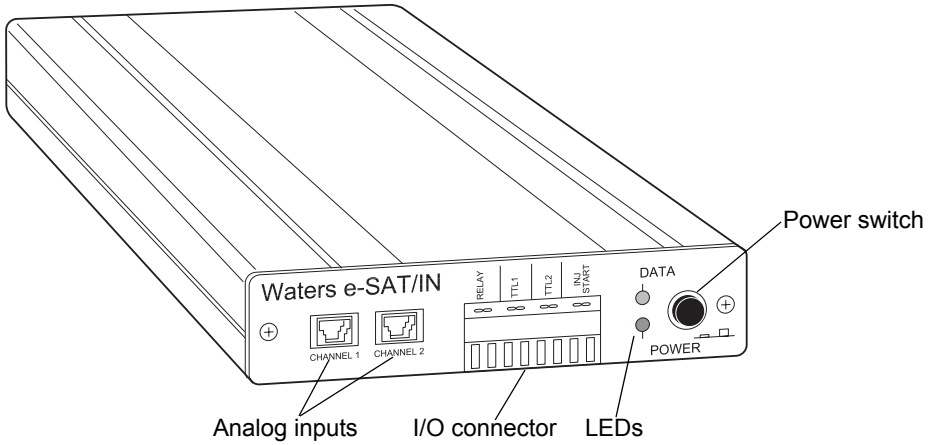
Acquiring data and controlling the detector with Empower software, by using the e-SAT/IN module instead of the Ethernet bus, requires connections between the following hardware:

- Laboratory acquisition and control environment (LAC/E) module (LAC/E^{32} Acquisition Server or busLAC/E card)
- Ethernet satellite interface (e-SAT/IN) module

e-SAT/IN module

The Waters e-SAT/IN module shown in the figure below translates analog signals from devices such as the detector into digital form. It then transmits these digital signals to the busLAC/E or LAC/E^{32} card installed in the Empower workstation.

e-SAT/IN module (front panel)



To connect the detector to the Empower workstation

Caution:

- The e-SAT/IN module does not have a power switch. Always disconnect the power cord at either the wall outlet or the power supply before attaching or removing the power connection to the e-SAT/IN module.
- To ensure proper startup of the e-SAT/IN module, do not power-on the module until you perform all procedures in the *Waters e-SAT/IN Module Installation Guide*. Improper startup can damage the unit and void the warranty.

Connect the detector to the e-SAT/IN module. (See page 2-21.)

Connecting the detector to the e-SAT/IN module

The e-SAT/IN module connects to the detector through the B (inputs and outputs) terminal on the rear panel of the detector, as shown in the figure below.

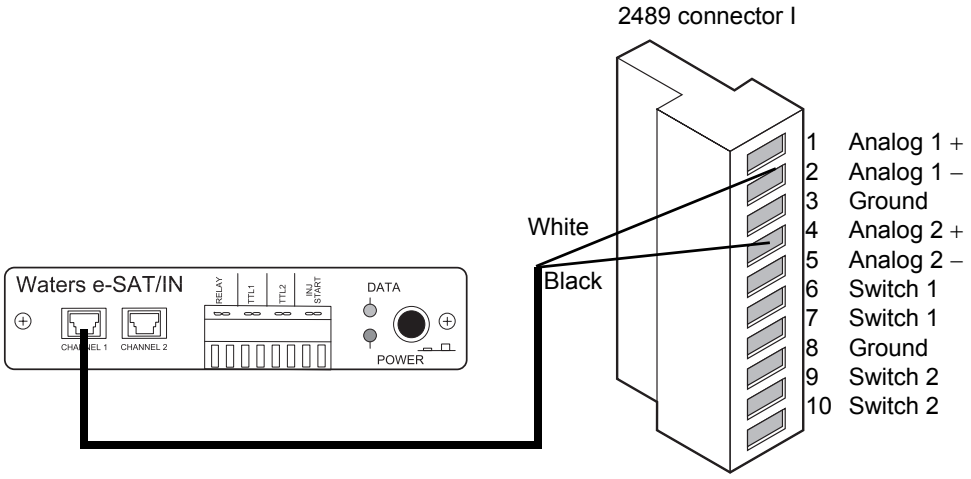
To connect the detector to the e-SAT/IN module

1. Using the electrical insulation stripping tool, strip about 3 mm (1/8 inch) from one end of the e-SAT/IN 9-pin connector, exposing the white and black wires.

2. Connect the other end of the cable to the Channel 1 or Channel 2 connectors on the front panel of the e-SAT/IN module.

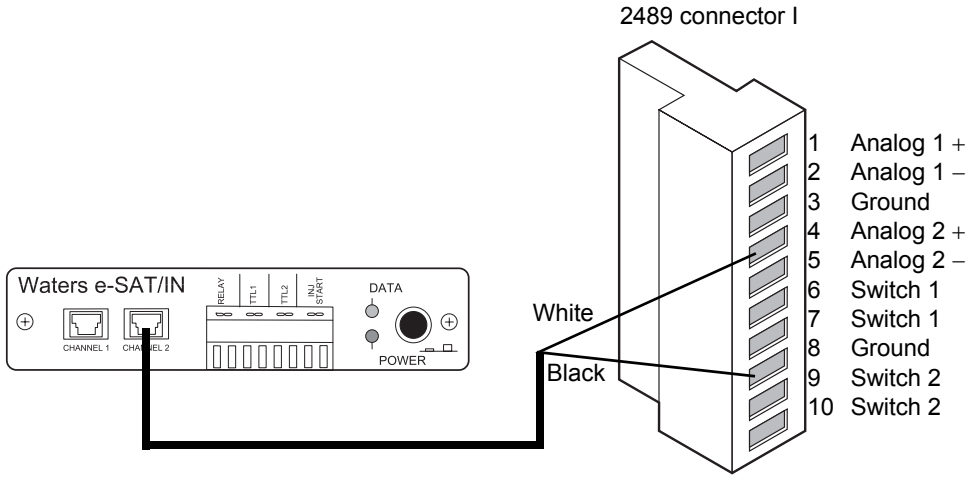
3. For Channel 1,
   - connect the white wire to pin 1 on I (Analog 1 +).
   - connect the black wire to pin 3 on I (Ground).

e-SAT/IN module channel 1 connection to detector



4. For Channel 2,
   - connect the white wire to pin 4 on I (Analog 2 +).
   - connect the black wire to pin 8 on I (Ground).

e-SAT/IN module channel 2 connection to detector



5. Configure the serial port for the e-SAT/IN module as described in the *Empower 2 Installation and Configuration Guide*.

The following table summarizes the detector connections to the e-SAT/IN module.

Detector connections to a e-SAT/IN module

2489 detector (I)	e-SAT/IN connector
Pin 1 Analog 1 + (white)	Channel 1 or 2
Pin 3 Ground (black)	
Pin 4 Analog 2 + (white)	Channel 1 or 2
Pin 8 Ground (black)	

Connecting the detector to a 745/745B/746 data module

You can connect the detector to a Waters 745/745B/746 data module using the analog output connector on the rear panel of the detector. The analog

connector provides 2-V output that is scaled to the AUFS sensitivity setting and the voltage offset setting.

Caution: To prevent oversaturation of the signal from the detector to the integrator, do not exceed the input voltage rating of the integrator.

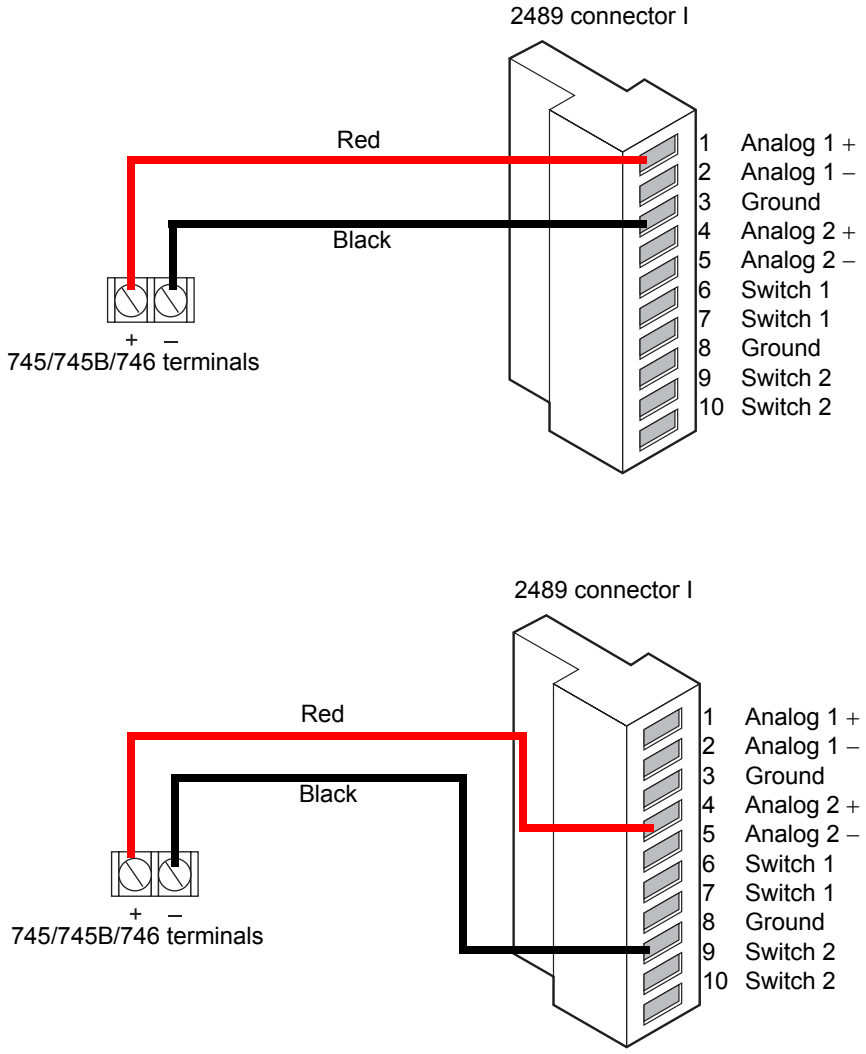
To send the analog output signal from the detector to the data module, use the cable provided in the 2489 Detector Startup Kit to make the connections summarized in the table below and illustrated in the figure below.

Detector connections to a data module

2489 detector (I)	745/745B/746 terminal
Pin 1 Analog 1 + (red)	+
Pin 3 Ground (black)	–
Pin 4 Analog 2 + (red)	+
Pin 8 Ground (black)	–

To minimize the chance of creating a ground loop that can adversely affect measurement, connect the shield of the cable to the chassis ground at one end only.

Data module connections to detector channels A and B



Connecting the detector to a chart recorder

Recorder signal

The A and B terminals on the rear panel of the detector provide 2-V analog output signals that you can use to connect the detector to a chart recorder.

To send 2-V signals from the detector to a chart recorder, use the cable provided in the 2489 Detector Startup Kit to make the connections summarized in the table and illustrated in the figure that follows it.

Detector connections to a chart recorder

2489 detector (B Inputs and outputs)	Chart recorder terminal
Pin 1 Analog 1 +	+
Pin 3 Ground	–
Pin 4 Analog 2 +	+
Pin 8 Ground	–

To minimize the chance of creating a ground loop that can adversely affect measurement, connect the shield of the cable to the chassis ground at one end only. For connection to other data systems, a cable can help remove the incidence of ground loops.

Tip: The detector is optimized for use with the 2-V analog output.

Connect the detector to a chart recorder using the 2-V analog connection as shown in the following figures.

Chart recorder 2-V output connections on the detector

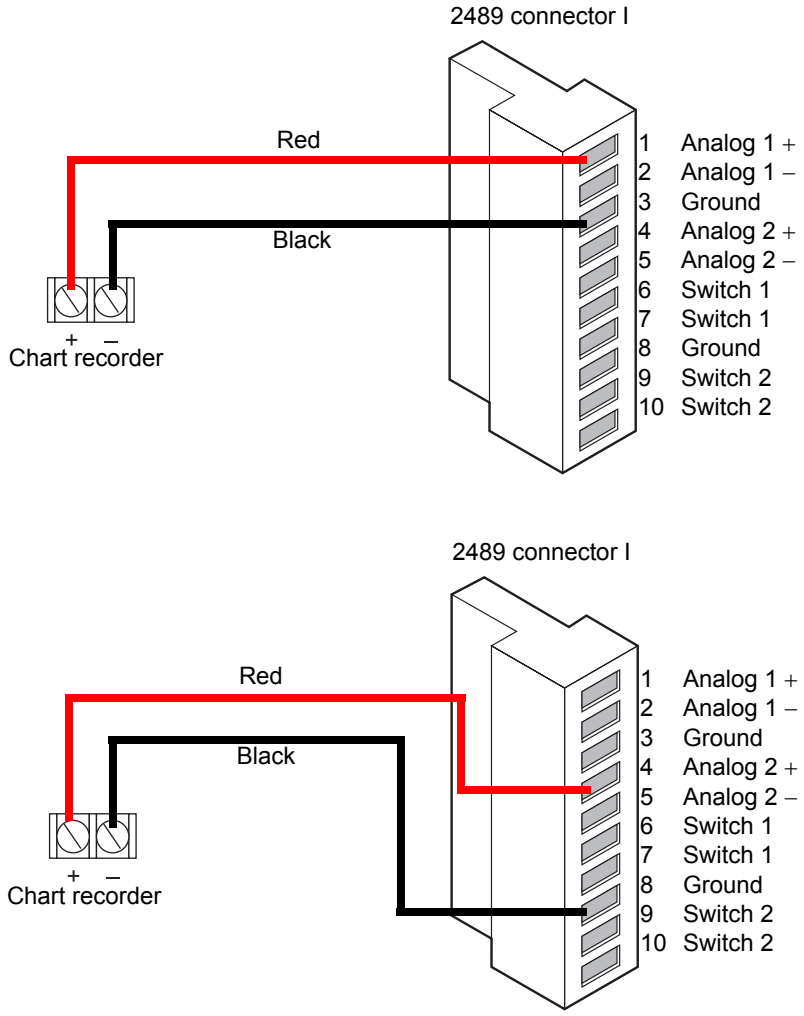


Chart-marks

You can generate a chart-mark from the front panel of the detector. A chart-mark signal results from one of these actions or events:

- You press the Chart-mark key on the detector keypad.
- You program a timed event to generate a chart-mark.
- Signal is received from one of the chart-mark inputs on the analog connector.

Connecting the detector to the Waters 600 series pump

To connect the detector to the pump, locate the detector in a position that satisfies the site requirements on page 2-3.

Plumbing connections

Make line connections as described in "Making plumbing connections" on page 2-6.

Lamp on/off connections

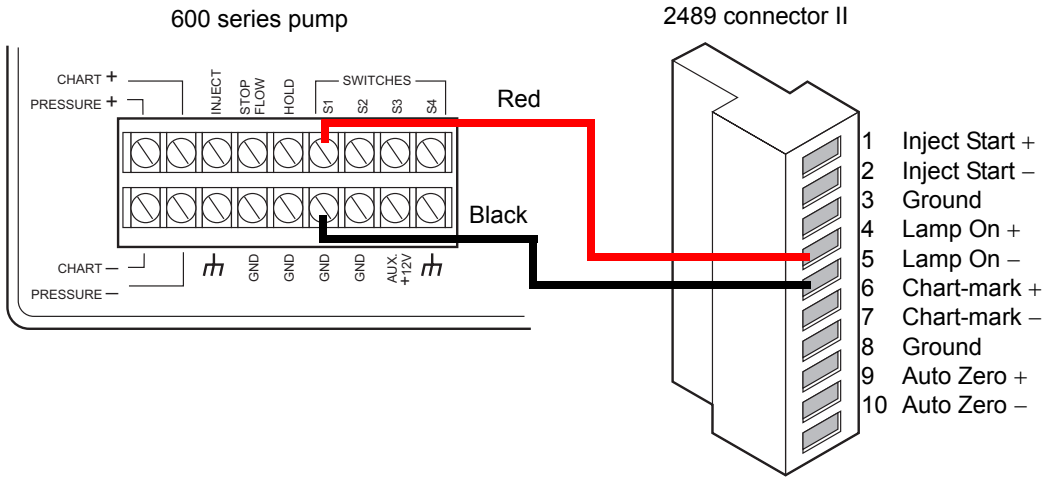
In addition to making the connections described below, you must configure the lamp on/off signal at the front panel. You must change the default from Ignore to High or Low. See the discussion page 3-23 for more information.

Using signal cable, make the connections between the pump controller and the detector that are summarized in the table and shown in the figure below.

Pump and detector lamp on/off connections

2489 detector (II)	600 series pump terminal
Pin 4 Lamp On/Off +	S1, S2, or S4
Pin 5 Lamp On/Off –	GND

Pump lamp on/off connections



Auto-zero connections

To make auto-zero connections between the detector and the pump

1. Using a signal cable, make the connections between the detector and the pump that are summarized in the table and illustrated in the figure below.
2. Program the pump to provide a pulse output on the applicable switch (S1, S2, or S4) at the beginning of each run.

   See also: *Waters 600E Multisolvent Delivery System User's Guide.*

Pump and detector auto-zero connections

2489 detector (II)	600 series pump terminal
Pin 9 Auto Zero +	S1, S2, or S4
Pin 10 Auto Zero –	GND (one of four)

Pump auto-zero connections

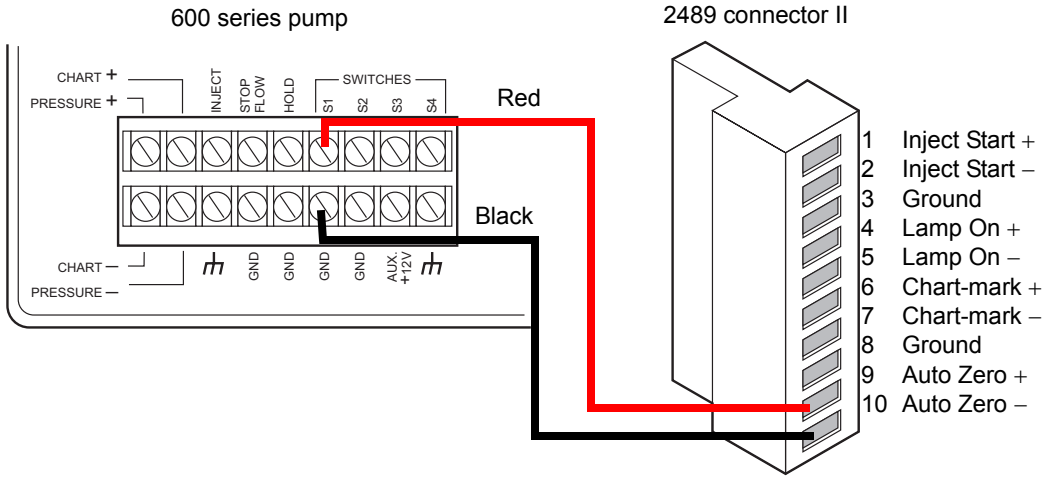


Chart-mark connections

To make chart-mark connections between the detector and pump

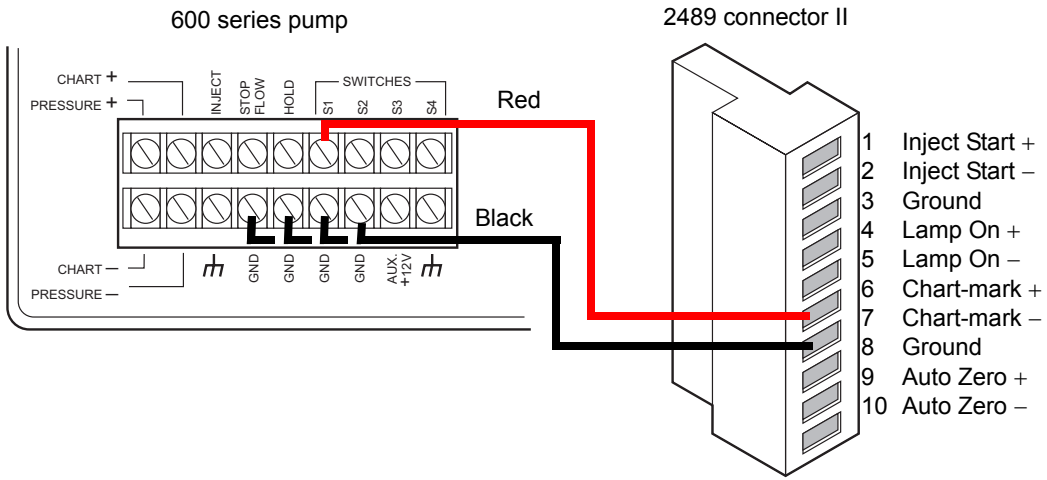
1. Using a signal cable, make the connections between the detector and the pump that are summarized in the table and illustrated in the figure below.
2. Program the pump to provide a pulse output on the selected switch at the beginning of each run.

   See also: *Waters 600E Multisolvent Delivery System User's Guide.*

Pump and 2489 detector chart-mark connections

2489 detector (II)	600 series pump terminal
Pin 6 Chart-mark +	S1, S2, or S4
Pin 7 Chart-mark –	GND (one of four)

Pump chart-mark connections



Inject start connections

Tip: If the detector is connected to an Empower data system, use the Inject Start connections to initiate the start of data acquisition.

To make inject start connections between the pump and the detector to start a method

1. Using a signal cable, make the connections between the detector and the pump that are summarized in the table and illustrated in the figure below.
2. Program the pump to provide a pulse output on the selected switch at the beginning of each run.

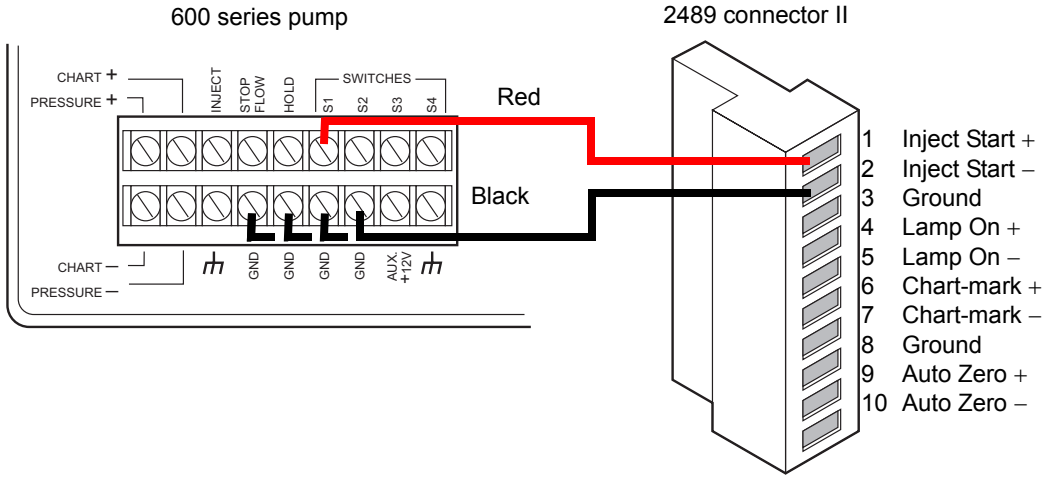
   See also: *Waters 600E Multisolvent Delivery System User's Guide.*

Pump and detector inject start connections

2489 detector (II)	600 series pump terminal
Pin 1 Inject Start +	S1, S2, or S4[a]
Pin 2 Inject Start –	GND (one of four)

a. You can also connect the pump inject pin to the Pin 1 Inject Start + on the detector, and the inject ground pin to the Inject Start – on the detector.

Pump inject start connections



Connecting the detector to the Waters 717plus autosampler

The Waters 717plus autosampler signals the start of an injection through a contact closure signal on its Inject Start terminals. You can use this contact

closure signal to command the detector to perform an auto-zero at the start of an injection.

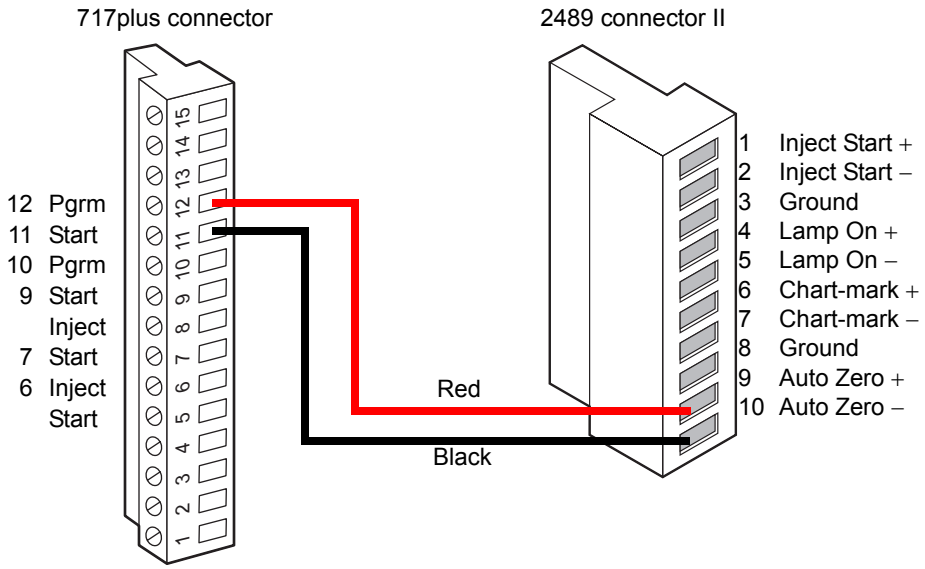
Auto-zero connections

To auto-zero the detector at the start of an injection, make the connections described in the table and illustrated in the figure below. Use any available pair of Inject Start terminals on the autosampler.

Autosampler and detector auto-zero connections

2489 detector (A inputs)	717plus autosampler terminal
Pin 9 Auto Zero **+**	Inject Start **+** (any one of three paired with –)
Pin 10 Auto Zero –	Inject Start – (any one of three paired with **+**)

Autosampler auto-zero connections to the detector



Inject start connections

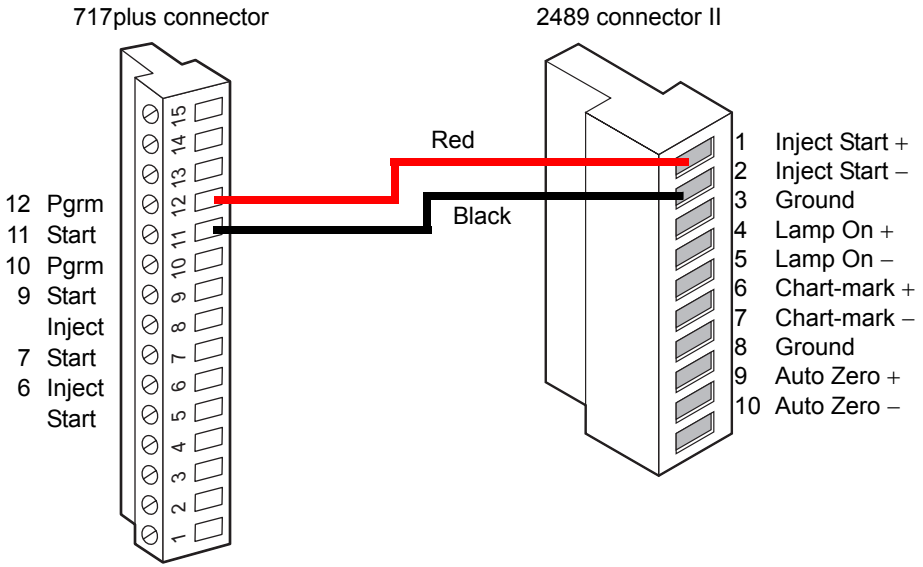
You can also use the Inject Start connections on the autosampler to connect to the Inject Start signals on the detector to program the start of the active method.

To program an inject start, make the connections described in the table and illustrated in the figure below. Use any available pair of Inject Start terminals on the autosampler.

Autosampler and detector inject start connections

2489 detector (II)	717plus autosampler terminal
Pin 1 Inject Start +	Inject Start + (any one of three paired with +)
Pin 2 Inject Start –	Inject Start – (any one of three paired with –)

Detector inject start connections



Connecting the detector to a fraction collector

The detector can trigger a fraction collector based on these things:

- Timed events (see "Timed events" on page 3-35)
- Threshold levels (see "Threshold events" on page 3-37)

You can connect the fraction collector to one of the detector's two programmable switches (SW1 or SW2), and program the timed event, threshold, or ratio at the front panel.

You can also connect the fraction collector to trigger a chart-mark event input each time a tube is changed at the fraction collector.

The table below indicates the detector-to-fraction collector and autoinjector-to-fraction collector connections.

Detector connections to the fraction collector

2489 connection	Fraction collector
I Pin 3 Ground	Pin 1 Detector In –
II Pin 6 Chart-mark +	Pin 10 Event Marker +
II Pin 7 Chart-mark –	Pin 9 Event Marker –
I Pin 6 SW1	Pin 7 External Count In +
I Pin 8 Ground	Pin 8 External Count In –
2695 separations module/717plus autoinjector	
Inject Start +	External Start In +
Inject Start –	External Start In –

See also: Documentation provided with your fraction collector for complete.

3 Preparing the Detector

Contents:

Topic	Page
Initializing the detector	3-2
Using the operator interface	3-4
Scanning spectra	3-42

After you install the detector, you can set it up and operate it as a stand-alone instrument or as part of a an Empower or MassLynx data system.

- As a stand-alone instrument – You can use the detector as a stand-alone detector within a system, or with any fluid-handling unit, injector, integrator, or data system. You can program the front panel of the detector for stand-alone operation, except when it is in remote mode (see page 3-26).
- As part of an Empower or MassLynx system – Configure the detector with either data system to control and collect digital data. To do so, follow the instructions in the system's online Help.

Requirement: To ensure accurate operation, and before pumping mobile phase or solvent through the flow cell, be sure to perform the procedures on page 3-27.

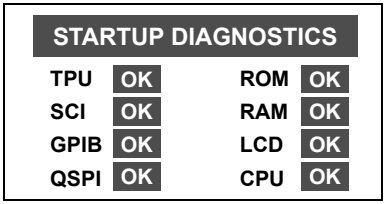
Initializing the detector

Before you power-on the detector, be sure the connector from the rear panel to the power source is properly installed.

To power-on the detector, press the On/Off switch located on the front, lower-right corner of the unit.

At startup, the detector beeps three times and runs a series of startup diagnostic tests. If it passes all startup diagnostic tests, the Startup Diagnostics screen appears, with "OK" following each test.

Startup Diagnostics screen



After it displays the Startup Diagnostics screen, the detector displays the following series of messages, in sequence, a process that takes about five minutes:

- Initializing grating
- Initializing system

- Lighting lamp
- Warmup time left: *n* minutes
- Homing optical filter
- Searching for 656 nm
- Optimizing system performance
- Finding calibration peaks
- Restoring last setup
- Completing initialization

When initialization ends, the detector displays the absorbance screen shown (see page 3-4). The sections "Using the keypad" on page 3-7 and "Navigating the user interface" on page 3-12 provide more information on this and subsequent screens.

Tip: For normal use, allow the detector to warm up for at least 30 minutes before operating.

Diagnostic test failure

If one or more of the internal startup diagnostic tests show a failing result, the detector beeps and displays an error message. For serious errors, it displays the word "Error", in brackets (<Error>), in place of the run-time absorbance on the absorbance screen.

See page 5-2 for a list of startup diagnostic test failures, error messages, and recommended recovery actions. See page 5-21 for hardware-related causes of startup diagnostic test failure and corrective actions.

Idle mode

When the detector is successfully started, it defaults to idle mode (see the figure "2489 detector idle mode screen" on page 3-4). When it is not performing any function requiring the shutter to be open (local methods, scans, noise test, and so on), the shutter is closed, and the detector remains in idle mode with the lamp lit. The Closed Shutter limits unnecessary UV light from reaching the detector's optics bench.

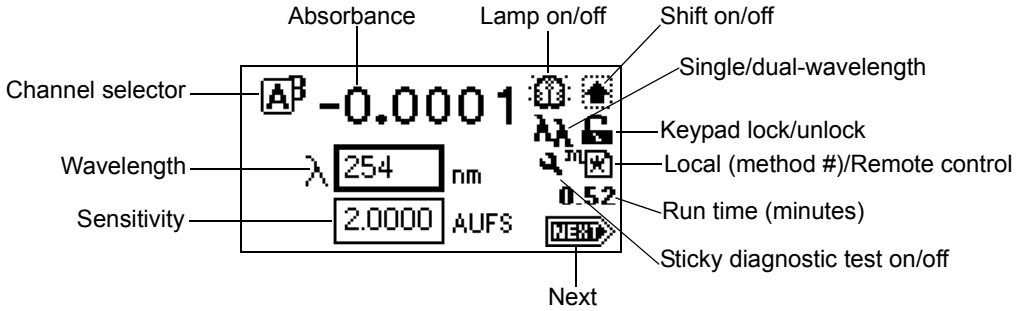
2489 detector idle mode screen

Using the operator interface

Using the display

The detector employs a 128 × 64-bitmap graphic display and a 24-key membrane keypad for the operator interface. After the startup diagnostic tests are run successfully, the detector displays the absorbance or home screen.

Detector absorbance screen



You can recall the absorbance screen at any time by pressing the HOME key. When you first use of the detector, the absorbance screen shows the factory-set defaults. Afterward, the absorbance screen shows the settings displayed before the detector was last powered-off. The absorbance screen continues to change as the run continues.

The detector monitors absorbance of one or two wavelengths in real time while allowing you to modify all the parameters discussed in the table below. You can use the A/B key to toggle between absorbance screens for channels A and B.

Absorbance and message icons

The absorbance screens and message screens in the detector program display the icons or fields shown in the figure on page 3-4 and described in the table below. For a list of ranges and defaults for the function icons and fields described in this table, see the table titled "Primary and secondary function (method) parameters" on page 3-18.

Caution: Changing the sensitivity (AUFS) setting affects the 2-V output. For example, 1 AU gives 0.5 AU/V, and 2 AU gives 1 AU/V.

Absorbance and message screen icons

Icon or field	Icon/field name	Function
Field requiring entry	Sensitivity or AUFS	Selects the chart sensitivity in absorbance units full-scale (AUFS) for the selected channel (Ethernet signal is not affected).
Field requiring entry	Wavelength	Selects the wavelength monitored on the selected channel. In single-wavelength mode, you cannot independently control wavelength on channel B.
	Channel selector	Changes the channel when you press A/B. The icon of the selected channel overlaps that of the other channel.
	Channel On	Displays ON A or ON B for the channel a timed or threshold event is programmed on.
	Channel trace	When you press TRACE, displays only the channel being viewed.
Numerical field	Absorbance	Displays current absorbance for the selected channel.
	Lamp on	Indicates the lamp is turned on.
	Lamp off	Indicates the lamp is turned off.

Absorbance and message screen icons (Continued)

Icon or field	Icon/field name	Function
	Shift off	Blank = Shift off
	Shift on	Indicates the shift is on for one key press.
	Single-wavelength	Indicates the detector is operating in single-channel mode.
	Multiwavelength	Indicates the detector is operating in dual-channel mode.
	Keypad unlock	Indicates unrestricted keypad entry.
	Keypad lock	Indicates parameter changes are not allowed. The keypad locks when the instrument is under control of an external data system (remote mode only).
	Sticky diagnostic on	Indicates a sticky diagnostic setting is active. (See page 5-9 for an explanation of sticky diagnostic settings.)
	Local method number	Indicates the detector is not controlled by a data system. The icon's cursive "m" and the current method number or an asterisk (*) indicate current conditions are not stored as a method.
	Ethernet control	Indicates that the detector is controlled by a data system.
Numerical field	Run time (minutes)	Displays the time elapsed since you pressed Run, or since an inject-start signal was received.
	Next	Indicates that pressing Next displays additional screens.

Absorbance and message screen icons (Continued)

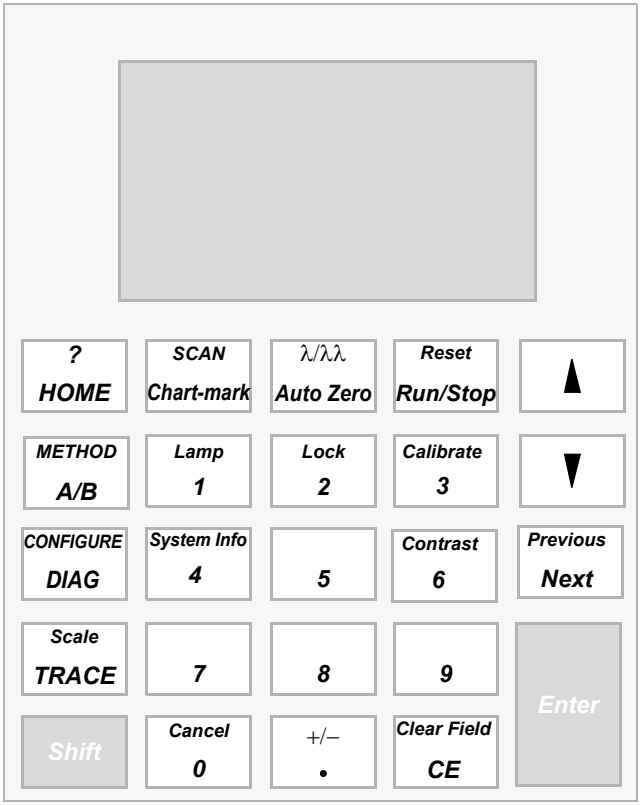
Icon or field	Icon/field name	Function
	Message screen icon.	Indicates an error message.
	Message screen icon.	Indicates a question.
	Message screen icon.	Indicates a warning message.
	Message screen icon.	Indicates information is being displayed.
	Message screen icon.	Indicates that you must standby.

Using the keypad

The detector keypad (shown below) consists of 24 keys providing

- full numeric entry (10 digits plus a decimal point).
- Enter, Shift, CE (Clear Entry), Next, and Help functions.
- s and t (used for navigation only; pressing s can also move the highlighted area to the left, t to the right).
- A/B for channel selection.
- navigation to specific screens (HOME, or absorbance; DIAGnostics; TRACE; CONFIGURE; METHOD; and SCAN).
- primary function keys (Chart-mark, Auto Zero, and Run/Stop).
- secondary function keys (Scale, Single or Dual-wavelength, Reset Clock, Lamp, Lock, Calibrate, System Information, Contrast, Previous, Cancel, +/–, and Clear Field).

Keypad



Primary function keys take effect immediately, with no further entry required. Secondary function keys require you to enter information into parameter fields and to press Enter for the functions to take effect.

Keys that appear in all caps (HOME, DIAG, TRACE, METHOD, CONFIGURE, and SCAN) directly invoke a function from most screens.

For numerical entries from 1 to 9 on choice lists or menus, enter the number corresponding to the desired item, and then press Enter. For the number 10, select 0, and then press Enter. To go to the end of a choice list, select •. For entries numbered 11 or 12, scroll to the desired item on the choice list, and then press Enter.

The table below presents the functions of the primary and secondary keys.

Detector keypad description

Key	Unshifted	Shifted
? HOME	HOME – Displays the absorbance screen containing the icons, and the Wavelength and AUFS fields.	? – Displays context-sensitive help when available.
SCAN Chart-mark	Chart-mark – Causes a momentary pulse to the analog output (A and/or B, depending upon the current settings). This key has no effect if chart-mark is disabled on both channels.	SCAN – Displays the list of options for generating and manipulating spectra.
λ/λλ Auto Zero	Auto Zero – Sets the absorbance offset so that the analog output (A and/or B, depending on the current settings), reads 0 AU. You can enable or disable the auto-zero function from the third absorbance screen (see the figure on page 3-18).	λ/λλ – From the absorbance screen, use this key to toggle between single- and dual-wavelength modes. The current mode is indicated by an icon on the display.
Reset Run/Stop	Run/Stop – Starts or stops (freezes) the run clock. (The elapsed time appears near the lower-right corner of the absorbance screen.) Initiates scans.	Reset – Resets the detector run clock to 0 minutes. Returns the detector to initial conditions for the current method.
▲ ▼	Arrow keys – On screens with entry fields (edit, check box, or choice list), the border of the active field is thickened (highlighted). Using the arrow keys, you move the highlighted board to the field you want to activate. (Up moves up or left; Down moves down or right.) On screens with scrollable lists, these keys move the highlighted border up (toward the beginning of the list) or down (toward the end). For other screens, special instructions apply to the use of the Up and Down arrow keys (for example, the Display Contrast screen).	

Detector keypad description (Continued)

Key	Unshifted	Shifted
Previous / Next	Next – Displays a screen with additional options related to a current screen. Repeatedly pressing this key brings back the screen on which you started. On most screens where this key is active, the NEXT arrow appears in the lower, right-hand corner of the display.	Previous – When the Next key is available, use Previous to navigate through the screens in the reverse order.
METHOD / A/B	A/B – On screens with the A/B icon in the upper, left-hand corner, this key toggles between channel A and channel B parameters.	METHOD – Displays the list of options for creating and clearing timed and threshold events, storing, retrieving, and resetting methods.
CONFIGURE / DIAG	DIAG – Displays the choice list of diagnostic tests.	CONFIGURE – Displays the first Configuration screen.
Scale / TRACE	TRACE – Displays the absorbance monitor trace for channel A or B.	Scale – When the wavelength trace or spectrum screen is visible, this function permits modification of the display range in the X (time or wavelength) and Y (absorbance) dimensions.
Shift	Shift – Enables the shifted functions (identified by the text at the top of most keys). The shifted state is temporary and resets after the next keystroke.	
0-9	0-9 – Enters a numeral, from 0 through 9, into a current field. Also positions the cursor at the corresponding entry in a list (0 = tenth item). Selects the corresponding number from a choice list.	0-9 – See descriptions for specific shifted numeric keys.

Detector keypad description (Continued)

Key	Unshifted	Shifted
Lamp *1*	1 – See 0-9, above.	Lamp – Displays a lamp use statistics for the currently installed lamp and provides the means for you to turn the lamp on or off. The current state of the lamp is indicated by an icon on the absorbance screen.
Lock *2*	2 – See 0-9, above.	Lock – Use from the absorbance screen, to lock or unlock the keypad. The lock prevents inadvertent changes to detector settings. The current lock state is indicated by an icon on the absorbance screen.
Calibrate *3*	3 – See 0-9, above.	Calibrate – Initiates wavelength calibration.
System Info *4*	4 – See 0-9, above.	System Info – Displays system information including firmware version and instrument serial number.
Contrast *6*	6 – See 0-9, above.	Contrast – Permits contrast adjustment (viewing angle) of the liquid crystal display.
Cancel *0*	0 – See 0-9, above.	Cancel – In some modes, backs out of a prompt without completing the task. The word “Cancel” appears as a cue in the lower, right-hand border of the message text.

Detector keypad description (Continued)

Key	Unshifted	Shifted
+/– •	• – Enters a decimal point. Also positions the cursor at the last entry in a list.	+/– – Some edit fields accept negative-number entries. Use this function to invert the sign of the number in an active field.
Clear Field *CE*	CE – Clears an editing change, and returns the contents of a field to its previous value. Sets the value to a unique word for some fields. For example, in the voltage offset diagnostic display, you can enter either a numeric offset value or press CE to change it to OFF.	Clear Field – Clears the current entry field before you enter the desired values.
Enter	Enter – Completes the entry in an edit field. Also advances the active field, as if the Down arrow had been pressed (except after editing the wavelength on the absorbance screen). Press Enter to acknowledge error messages and other prompts. In these cases, the word "Enter" appears as a cue in the lower, right-hand border of the message text.	

Navigating the user interface

To operate the detector

1. Press the Enter key or the up and down arrow keys to navigate among editable fields on a display.

   Tip: A thickened border highlights the active field.

2. Press Enter to advance the active field once you complete an entry.

3. When you make an error, press CE (Clear Entry) to undo any changes and return to the active entry field.

   Tip: An active field containing a choice list is indicated by a numeral to the right of the field and within the thickened border.

4. To display a choice list, press Enter. Then take one of these actions:
   - Press the corresponding number key to select an item immediately.
   - Use the up and down arrow keys to scroll through the list, and then press Enter.

   Tip: You can press the numeral that corresponds to a desired choice without first pressing Enter.

Rule: The Up and Down arrow keys do not incrementally increase or decrease numerical field entries. Use the numerical keypad for that purpose.

Navigating to and from the absorbance screen

Pressing HOME from most screens causes the absorbance screen to appear. From the absorbance screen, you can access several secondary functions. To move to the secondary function screens of the absorbance screen, press Next. These are the secondary functions:

- Analog output specifications
- time-constant
- Absorbance offset
- Voltage offset
- Chart polarity
- Enable/disable inputs
- Enable/disable external events

Tip: The parameters you enter into the secondary function fields become part of the current method conditions and are stored when you store the method (see page 3-34).

In single-wavelength mode, the detector displays three additional screens, labeled 2 of 4, 3 of 4, and so on. In dual-wavelength mode, the detector displays four additional screens, labeled 2 of 5, 3 of 5, and so on (see page 3-18).

Setting up a run

Once you press HOME, causing the absorbance screen to reappear, and you select a wavelength mode (λ or λλ), you can set up the detector for a run. In

addition to wavelength mode, you must program the following parameters before beginning a run:

- Operating wavelength
- Sensitivity
- time-constant
- Analog output sensitivity

Depending on other functions that you can perform during a run, several other parameters can require programming. See page 3-14 and the table on page 3-18 for the function descriptions, fields, screen number, type of function, display units, allowable ranges, and the default settings for the absorbance screen and the secondary function screens.

Primary and secondary functions

You can access the following functions directly from the absorbance screen or by pressing the Next key on that screen.

Detector functions

Function	Description
Wavelength	Defines the operating wavelength for the channel.
AUFS (absorbance units full-scale)	Defines the relationship between the absorbance and the output voltage. The output voltage reaches full scale (2 V) when the current absorbance attains the AUFS value. **Caution:** Changing the sensitivity (AUFS) setting affects the 2-V output.
Analog out (single λ)	You can chart the analog connections for the following parameters: • Absorbance – Charts the current absorbance of a selected channel's analog output, 2-V connector (scaled to the AUFS setting for that channel) and adjusts the absorbance value according to the voltage and absorbance offsets for that channel. **Example:** For the 2-V output with 0 used for both the voltage and absorbance offsets; Volts out = Absorbance × 2 V/AUFS

Detector functions (Continued)

Function	Description
Analog out (dual λ)	In addition to the selections for single λ above, you can chart the same parameters on the other channel, at a different wavelength, and you can chart the following parameters: • MaxPlot – Charts the absorbance of multiple compounds with different absorbances at two different wavelengths on a single data channel. Scaling for MaxPlot is the same as for Absorbance (above) except that the charted absorbance is the larger of the two absorbances as measured on channel A and channel B. The detector uses the AUFS, absorbance offset, and voltage offset of the selected channel regardless of which absorbance (channel A or channel B) is larger. Volts out = Larger absorbance (A or B) × 2 V/AUFS (of selected channel) • RatioPlot (A/B) – Charts the ratio of absorbances at two wavelengths. Theoretically, the ratio is constant for a pure chromatographic peak and variable for an impure peak. This mode applies the three ratio parameters on screen 5 of 5 (see the figure below). – Minimum AU: This setting defines the minimum AU value for either wavelength (A or B) before the actual ratio is calculated. Both absorbance values (A and B) must be above this value or 0 V is charted. If both absorbances are above this value, the absorbances are divided (A/B) and charted. The output voltage is scaled proportionally to the ratio depending on the selected channel minimum ratio and maximum ratio settings. – Minimum ratio: An actual ratio equal to the minimum ratio value results in 0 V being charted.

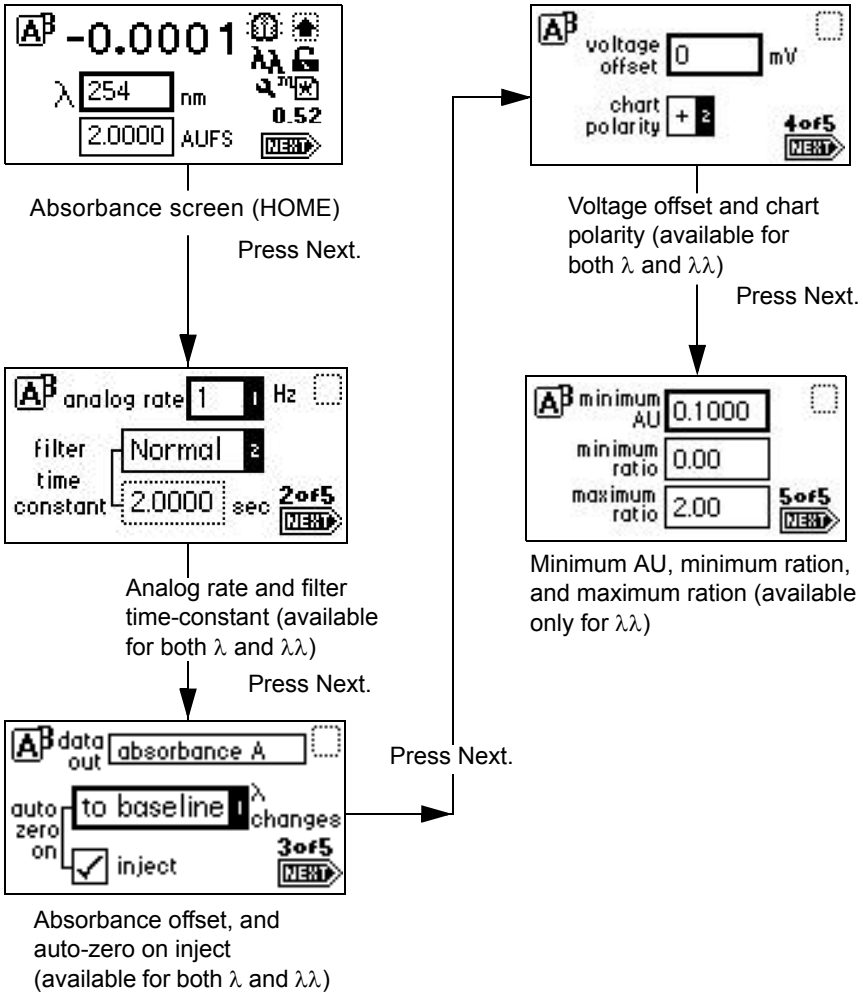
Detector functions (Continued)

Function	Description
	– Maximum ratio: An actual ratio equal to the maximum ratio results in a full-scale output of 2 V. Absorbance offset is ignored with this selection. For a RatioPlot, the actual voltage charted is Volts out = 0 V if Absorbance A and B < minimum AU Volts out = (Absorbance ratio – minimum ratio) × 2 V/(maximum ratio – minimum ratio) To ensure proper operation of the RatioPlot function, be sure the selected time-constants for both channels are set to the same value. • Difference Plot (A-B) – Charts the difference in absorbances at two different wavelengths. The scaling for the difference plot is identical to the Absorbance selection above, except that the charted absorbance is the difference (subtracted) in value of the two absorbances measured on the A and B wavelengths. The detector uses the AUFS, absorbance offset, and voltage offset of the selected channel for scaling. Volts out = Absorbance difference (A – B) × 2 V/AUFS (of selected channel)
Filter time-constant	Adjusts the noise filter (time-constant) to achieve the optimum signal-to-noise ratio without changing the sensitivity setting. See the discussion "Filtering noise" on page 1-7 in for more information.
Voltage offset	Adjusts the charted analog output signal. Entered in millivolts, this function adjusts the 2-V signal by the entered value. This function is useful for making minor adjustments and for rendering null any offset between the detector and a connected external data system.
Chart polarity	Inverts the charted chromatogram. Entering the plus sign (+) produces a normal chromatogram; entering the minus sign (–) produces an inverted chromatogram.

Detector functions (Continued)

Function	Description
Auto-zero on inject	Selected by default, this parameter results in an auto-zero each time the detector receives an inject-start signal via contact closure, Ethernet, or through the front panel. You can disable this parameter by pressing any numerical key.
Auto-zero on λ changes	This parameter results in an auto-zero each time a wavelength change is requested. If you disable this function, significant changes in measured absorbance can occur after each wavelength change. Selecting “to zero” sets the signal level to zero. Selecting “to baseline” maintains the previous baseline level when the wavelength change is made. The default is “to zero.”

Secondary functions of the absorbance screen



Primary and secondary function (method) parameters

Function	Screen	Type	Units	Range	Default
λ (Wavelength)	1 (Absorbance screen)	Numeric	nm	Integer 190 nm to 700 nm	254 nm
AUFS	1	Numeric	AUFS	0.0001 to 4.0000	2.0000

Primary and secondary function (method) parameters (Continued)

Function	Screen	Type	Units	Range	Default
Analog rate	2 (of 4) or 2 (of 5)	Choice	Hz	(λ): 10, 20, 40, 80 (λλ): 1 or 2	10 1
Filter time-constant	2 (of 4) or 2 (of 5)	Numeric	Sec	Slow Normal Fast Other: (λ): 0.0125 to 5.0 (λλ): 0.5 to 5.0 0 to disable filtering	Normal (for Other:) 1.000
Data out (single λ)	3 (of 4)	Choice	None	Absorbance A	Absorbance A
Data out (dual λ)	3 (of 5)	Choice	None	Absorbance A Absorbance B Maxplot A, B Difference A-B Ratio A/B	Absorbance A
Auto-zero on inject	3 (of 4) or 3 (of 5)	Check box	None	Selected/ Not selected	Checked
Auto-zero on λ changes	3 (of 4) or 3(of 5)	Choice	None	To baseline To zero Disable	To zero
Voltage offset	4 (of 4) or 4 (of 5)	Numeric	mV	Integer –2000 to 2000	0
Chart polarity	4 (of 4) or 4 (of 5)	Choice	None	+ –	+
Minimum AU	5 (of 5)	Numeric	AU	0.0001 to 4.0000	0.1000
Minimum ratio	5 (of 5)	Numeric	None	0.00 to 999.99	0.00
Maximum ratio	5 (of 5)	Numeric	None	0.00 to 999.99	2.00

Operating the Trace and Scale functions

Using the Trace function, you can display an absorbance trace for the last *n* minutes (as many as 60) of detector operation.

- When you press the TRACE key, the detector displays the absorbance acquired over the last 30 minutes by default. It updates the trace once every 20 seconds.
- When you press the Scale key (Shift TRACE), the detector displays the scaled trace with T1 (ending time) displayed (–30 for the last 30 minutes) by default.

  You can change the ending time parameter to any number from 1 to 60. You can use the Scale function to "zoom" in on a particular section of the trace.

To display the Scale parameters after you press the Scale key

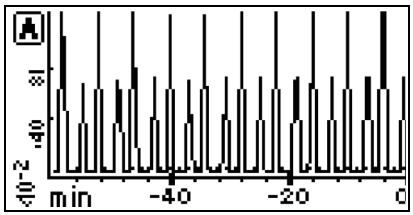
1. Press Next to display T2 (starting time). The default is 0.
2. Press Next again to display AU1 (starting or low absorbance). The default is Auto.
3. Press Next again to display AU2 (ending or high absorbance). The default is Auto.

   By entering appropriate times and absorbance numbers in the four scaling parameter boxes, you can zoom in on one section of the current absorbance trace:

   - For AU1 and AU2, press CE to reset to auto.
   - T1 represents the left-hand side of the trace, or ending time to be displayed. The default is –30.
   - T2 represents the right-hand side of the trace, or starting time. The default is 0.

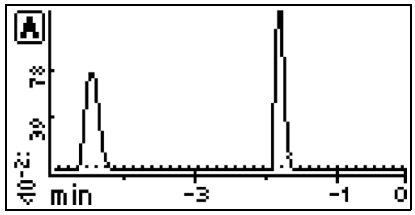
The figure below shows a 60-minute trace of continuous injections of caffeine and propyl paraben in 1:1 methanol/water.

Scaled trace of continuous injections, with T1 changed to –60



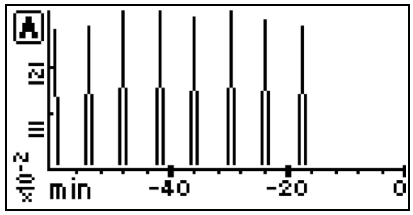
The figure below shows a five-minute scaled trace (or zoom) of the 60 minutes of continuous injections shown in the previous figure. T1 is changed to –5. T2 is changed to 0. AU1, and AU2 remain as "auto."

Scaled trace for 5 minutes of continuous injections, with T1 changed to –5



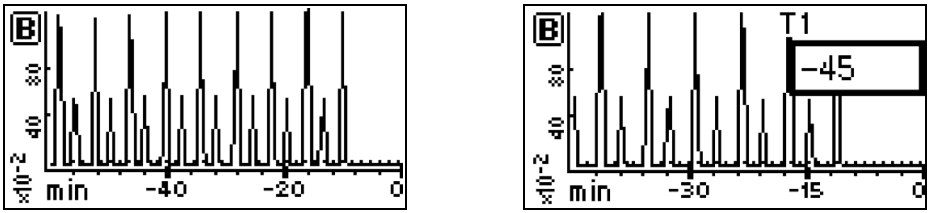
The figure below shows a 60-minute scaled trace similar to that shown in the preceding figure, with the starting absorbance or AU1 changed from auto to 1. T1 remains at 60. T2 remains at 0.

Scaled trace for 60 minutes of continuous injections, with AU1 changed to 1



The figure below shows a 60-minute trace on channel B scaled to the last 45 minutes. T1 is changed to –45.

Scaled trace changing T1 to –45



As you modify the output using the Scale function, the Trace function continues to display the detector output in real time on either or both channels.

Operating other detector functions

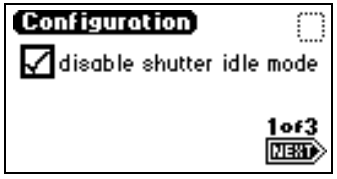
Configuring the detector

To change default configurations, use the Configuration screens.

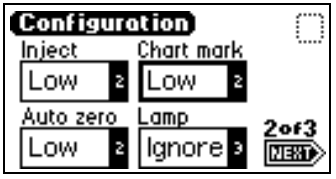
Press the CONFIGURE key (Shift DIAG). The first of three Configuration screens appears.

Tip: Other functions, such as specifying event inputs and enabling pulse periods, are activated from the Configuration screens.

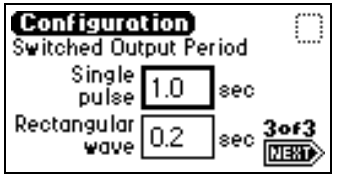
Configuration screens



Configuration screen 1 of 3



Configuration screen 2 of 3



Configuration screen 3 of 3

Requirement: To prevent acquisition of incorrect data when operating the detector in dual-wavelength mode under the control of the Empower software or a MassLynx system, you must select a data sampling rate of 1 point per second.

Configuring event inputs (contact closures)

You use the CONFIGURE key also to edit event input settings and specify switched output settings.

Using the Enter key and the numeric keypad (or the s and t keys) to select the appropriate entry, you can edit four fields on the second Configuration screen:

Tip: The default for Inject, Chart-mark, and Auto-Zero is Low; the default for Lamp is Ignore.

- Inject – You can specify the inject input to signal the start of a run. This event resets the run-time clock and applies initial method conditions immediately:
  - High – Start run when contact closure changes from off (open) to on (closed).
  - Low – Start run when contact closure changes from on (closed) to off (open).
  - Ignore – Do not respond to inject-start input.
- Chart-mark – You can specify the chart-mark input to create a chart-mark on channel A and/or channel B. You determine the response of the channel using the enable chart-mark function explained in the table on page 3-18 and shown in the figure on page 3-18.
  - High – Create chart-mark(s) when contact closure changes from off (open) to on (closed).
  - Low – Create chart-mark(s) when contact closure changes from on (closed) to off (open).
  - Ignore – Do not respond to chart-mark input.
- Auto-zero – You can configure the auto-zero input to auto-zero absorbance readings on channel A and/or channel B. You determine the response of the channel using the enable auto-zero function explained in the table on page page 3-18 and shown in the figure on page 3-18.
  - High – Auto-zero the channel when contact closure changes from off (open) to on (closed).
  - Low – Auto-zero the channel when contact closure changes from on (closed) to off (open).
  - Ignore – Do not respond to auto-zero input.

- Lamp – You can configure the lamp input level to turn the deuterium lamp on or off from an external device as follows:
  - High – Turn lamp off when contact closure is on (closed).
  - Low – Turn lamp off when contact closure is off (open).
  - Ignore – Do not respond to Lamp input.

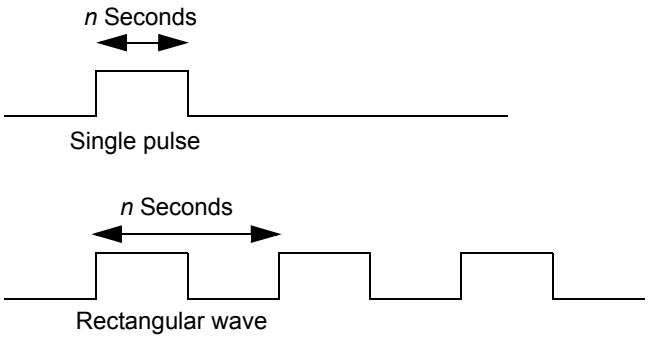
Setting pulse periods

You use the third Configuration screen (see page 3-22) to set pulse or signal width or to activate a pulse or rectangular wave on SW1 or SW2.

- Single pulse (in seconds) – If SW1 or SW2 is programmed to generate a pulse as a timed or threshold event, then the period of the signal (single pulse width) is as specified in this field (range is 0.1 to 60 seconds).
- Rectangular wave (in seconds) – If SW1 or SW2 is programmed to initiate a rectangular wave as a timed or threshold event, then the period of the signal (the width of one pulse period in a rectangular wave or pulse train) is as specified in this field (range is 0.2 to 60 seconds).

The figure below shows the difference between a single pulse and a rectangular wave.

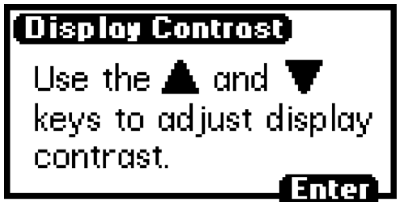
Setting the pulse period or signal width using SW1 or SW2



Setting display contrast

The Contrast function allows you to adjust the contrast of the detector display screen. When you press the Contrast key (Shift 6), the Display Contrast screen appears.

Display Contrast screen



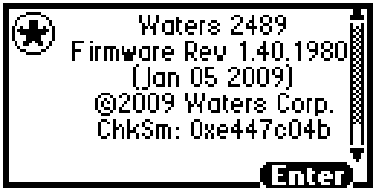
Use the s and t keys to adjust the contrast of the display.

Displaying system info

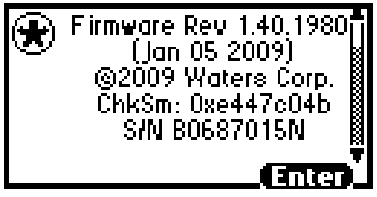
The System Info key (Shift 4) displays information about the detector including the serial number and the firmware version number.

Tip: Use the scroll bar to see all of the message. The Firmware Rev and ChkSm values shown here are examples only. They do not indicate the released version's information.

System info screen example



Start of message

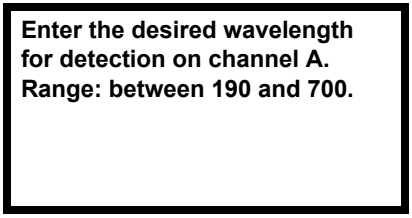


End of message

Using Help

The detector has limited context-sensitive help. When you press ? (Shift HOME) from a point in the program that has a Help screen associated with it, the appropriate screen appears.

Help screen



To exit the Help screen, press Enter. If online Help is not available for the function you are working on, pressing ? gets no response.

Operating the detector

Overview of detector operation

Tip: If you are operating the detector under the control of an external system, you can program any parameters not controlled by the external data system at the front panel of the detector before the external system takes control.

Recommendation: To prevent reabsorption of dissolved oxygen, run the solvent degasser continuously when operating the detector at wavelengths less than 230 nm.

Requirement: To maintain optimum system performance, be sure to replace the front, left-hand panel cover before resuming normal operation of the detector.

Operating modes

You can use the detector in either single or dual-wavelength mode over a range of 190 to 700 nm. The detector defaults to the mode of operation in effect when the instrument was last powered-off.

Stand-alone operation

When using the detector as a stand-alone instrument, you can store as many as five methods containing as many as 50 timed and 2 threshold events each. An asterisk in the method number field on the absorbance screen indicates current conditions, not a stored method. See page 3-34 for information on how to store a method.

Remote control

To operate under Empower or MassLynx control, the detector uses the Ethernet bus connector (see page 2-13).

To connect the detector to your HPLC system, see page 2-6.

When under the control of an external data system, the detector is operating under remote control conditions. The Remote Control icon, incorporating the letter “E”, appears on the absorbance screen (see the table on page 3-5).

See page 2-10 for more information on connecting the detector to an external system.

Verifying that the detector operates properly

After you have installed the detector, verify that it is operating properly by performing the procedures in this section.

Requirement: For complete validation procedures, you must obtain the Waters accuracy and linearity cuvette kits and system qualification tool for the detector. For spare parts details, see the Waters Quality Parts Locator on the Waters Web site’s Services & Support page.

Tip: Before you pump solvent or mobile phase through the system, flush the lines with filtered, degassed, and sparged HPLC-grade methanol. Then pump your mobile phase, provided that there are no miscibility problems, at 1 mL/min for 15 minutes minimum.

Before you begin

Because the detector is shipped dry, you must pump solvent through the unit before its initial use.

Rule: To ensure accurate verification, be sure to start up the detector and follow steps 1 to 3 in this section and 1 to 4 in “Recording sample and reference beam energies” on page 3-28 before pumping any mobile phase or solvent through the flow cell.

To pump solvent through the unit before initial use

1. Connect the detector to a data system or chart recorder (see Chapter 2 for complete information on connecting the detector to external devices).

2. Power-on the detector.

   Result: The front panel displays a series of initialization messages for about 5 minutes. See page 3-2.

3. When initialization ends, the detector displays the absorbance screen.

4. Allow the detector to warm up for at least 30 minutes before operating.

   Tip: If the startup verification diagnostic tests fail, note the error message to determine the corrective action, and refer to Chapter 5.

Recording sample and reference beam energies

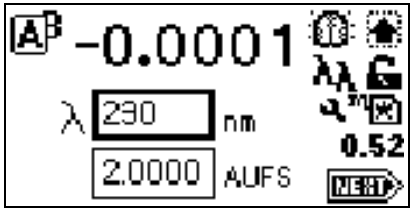
To determine baseline values on your detector for future reference and to monitor lamp aging (decreased lamp output energy). You must record the baseline sample and reference beam energies to compare against future readings. Use these baseline values to troubleshoot the detector to determine whether

- the solvent is contaminated.
- the flow cell is contaminated.
- the lamp needs to be replaced.
- there is an air bubble in flow cell.

To record the sample and reference beam energies

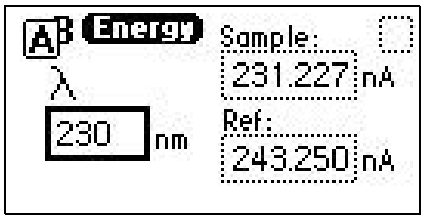
1. On the absorbance screen, use the arrow keys to highlight the λ field.

2. Enter 230 into the λ field, and then press Enter.



3. Press DIAG and then press 2, Sample & ref energy. The sample and reference energy diagnostic display appears.

Sample and reference energy diagnostic display



4. Record the numbers for later comparison.

   Rule: Run this procedure each time you change the detector lamp.

5. Flush the flow cell with approximately 30 to 60 mL of HPLC-grade methanol at 1 mL/min for 15 minutes, minimum, to clean the cell.

Make the plumbing connections described in "Making plumbing connections" on page 2-6, before beginning the peak response test.

Verifying peak response

This test evaluates the detector's peak response.

To run the test

1. On the absorbance screen, use the arrow keys to highlight the λ field.
2. Enter 254 into the λ field, and then press Enter.
3. Press Enter again to activate the sensitivity field.
4. Set the sensitivity to 2.0 AUFS.
5. Set the pump flow rate in your HPLC system to 1.0 mL/min.
6. Inject 1 µL acetone.

When the test is successful, your chart recorder or data system displays a peak.

Wavelength calibration

You can calibrate the detector, manually, from the keypad at any time during operation or if calibration arise errors during startup. You need not reboot the detector after a successful wavelength calibration.

To calibrate the detector manually

1. Press Calibrate (Shift 3) from the detector keypad.

   Result: A message appears asking whether you removed the cuvette and flushed the flow cell with a transparent solvent (Waters recommends methanol or water).

2. Press Enter to continue the calibration cycle or Cancel to invoke the absorbance screen without calibrating the detector.

   Result: After you press Enter, the detector cycles through the calibration procedure and briefly displays a series of initialization messages similar to those you saw at startup (see page 3-2).

   If calibration is successful, the detector beeps three times and displays the maximum error, in nanometers, of the calibration shift farthest from the last calibration.

3. Press Enter to complete calibration.

   Result: A “Calibration complete” message appears momentarily. Other messages such as “Optimizing system performance” and “Restoring last setup” can appear before the display returns to the absorbance screen.

   If calibration is successful, the error message (<Error>) that was displayed on the absorbance screen before you recalibrated the detector disappears.

4. If calibration is unsuccessful, retry, cycle power to the detector, or see Chapter 5.

Operating the detector in single-wavelength mode

The detector is optimized for single-wavelength operation, the default operating mode.

To invoke single-wavelength mode

1. From the absorbance (or HOME) screen, press the λ/λλ key (Shift Auto Zero) if the detector is in dual-wavelength (the wavelength icon shows λλ) mode.

   Result: The detector displays a message indicating it is switching to single-wavelength operation.

2. Enter the wavelength and sensitivity on the absorbance screen, as well as any secondary parameters and timed or threshold events.

   Caution: Changing the sensitivity (AUFS) setting affects the 2-V output.

3. Select a second sensitivity setting while in single-wavelength mode, by pressing A/B and specifying the appropriate AUFS values on the channel B screen.

   Result: A single-wavelength is tracked on channel A so that you can use channel B to monitor absorbance with an alternate AUFS setting, or to make the primary absorbance measurement using a specific AUFS value on channel A.

   Example: While operating in single-wavelength mode, specifying an AUFS value of 2.0000 on the second channel provides 1.000 V/AU on the channel B, 2-V output.

   The detector automatically engages the second-order filter for all wavelengths > 370 nm.

Operating the detector in dual-wavelength mode

You can operate the detector with expanded chart-out selections using dual-wavelength mode. In addition to absorbance offered with single-wavelength mode, dual-wavelength mode offers the following functions:

- Absorbance (A and B)
- MaxPlot
- RatioPlot (A/B)
- Difference plot (A-B)

Changing from single to dual-wavelength mode

To change from single to dual-wavelength mode

1. From the absorbance (or HOME) screen, when in single-wavelength mode (the wavelength icon shows λ), press the λ/λλ key (Shift Auto Zero).

   Result: A momentary message "Setting up dual-wavelength mode" appears.

2. Enter the wavelength to be monitored in the λ field, and then press Enter.
3. Enter the other operating parameters and timed or threshold events, if desired.
4. Press the A/B key to switch channels. The absorbance screen for the other channel appears.
5. Enter the operating parameters for monitoring the second wavelength, including timed and threshold events, if desired.

Tips:

- If both selected wavelengths are greater than 370 nm (+/– 1 nm), the detector applies the second-order filter to block unwanted UV light.
- If both selected wavelengths are less than 370 nm (+/– 1 nm), the detector removes the second-order filter.
- If the selected wavelengths bracket the 370 nm (+/– 1 nm) threshold, the detector does not apply the second-order filter and issues a warning message that any data collected for the wavelength above 370 nm can contain inaccuracies because of possible UV light interference (second-order effects).

Recommendation: In dual-wavelength mode, select a wavelength pair below or above 370-nm. If one or both of the selected wavelengths spans the 370 nm threshold, the detector beeps three times, and the warning message shown below appears. Because of possible UV light interference (second-order effects), you can observe additional peaks and inaccurate peak areas.

Dual-wavelength, 370-nm, threshold warning message

Obtaining a RatioPlot

The RatioPlot output, on one channel only (channel A), depends on the values you specify on absorbance screen 5 for minimum and maximum ratios. You must be operating the detector in dual-wavelength mode to obtain a ratio plot. The RatioPlot provides a plot of the ratio of the absorbances of two wavelengths from 0 V to 2 V. The minimum and maximum ratio parameters are measured ratio units, not absorbances. (See page 3-14.)

To obtain a RatioPlot

1. Be sure the detector is operating in dual-wavelength mode (see the previous discussion).
2. From the absorbance screen, press Next to reach screen 3 of 5.
3. In the Data out field, press 8, ratio A/B.
4. Press Enter to select the ratio plot.
5. Press Next until screen 5 of 5 appears.
6. Enter the minimum AU, and then press Enter.

   Tip: The minimum AU field contains a threshold value. The RatioPlot function does not plot if either wavelength does not exceed the minimum AU threshold.
7. Enter the minimum ratio for the RatioPlot, and then press Enter.
8. Enter the maximum ratio for the RatioPlot, and then press Enter.
9. Return to the absorbance screen by pressing the HOME key.

Obtaining a MaxPlot

The detector obtains a MaxPlot by monitoring absorbance at two selected wavelengths while plotting the maximum absorbance for each sample component.

To run a scan using the MaxPlot function

1. Be sure the detector is operating in dual-wavelength mode (see page 3-32).
2. From the absorbance screen, press Next to reach screen 3 of 5.
3. In the Data out field, press 5, MaxPlot A,B.
4. Press Enter to select the MaxPlot function.
5. Return to the absorbance screen by pressing the HOME key.

Programming timed events, threshold events, and methods

You can retrieve as many as five methods, which the detector references as numbers 1 to 5. An asterisk in the method number icon (see the table on page 3-5) tells you the current conditions are not stored. If you are using a stored method, the method number appears on the absorbance screen.

If you edit a parameter such as wavelength or AUFS, you are editing the current conditions (Method *), which can be stored as a method. You can store the method in one of the 10 available method storage slots, or you can replace the current method with one of the methods previously stored. When you recall a previously stored method, you replace the existing method conditions with those of the stored method.

The method number displayed in the absorbance screen is that of the retrieved method until you make a change. Any parameter change (for example, wavelength or AUFS) alters the current conditions so that the original recalled method is no longer in effect, causing the method number to change to an asterisk.

The operating parameters at the time of system shutdown are restored; however, any timed events or thresholds associated with the method are deactivated when power is restored. On startup, you always see an asterisk inside the method icon on the absorbance screen.

When the detector is operating under remote control—that is, control by Empower or MassLynx software—the remote icon incorporating the letter "E" appears (see the table on page 3-5).

Timed events

You can program as many as 50 timed events, to the nearest 0.01 minutes. As you enter timed events, each new event appends to the end of the timed-event list. If you enter a time that is not in sequence with the events entered previously, the timed-event list sorts automatically when you press Next. The detector allows programming of the timed events shown in the table below.

Caution: Changing the sensitivity (AUFS) setting affects the 2-V output. For example, 1 AU gives 0.5 AU/V, and 2 AU gives 1 AU/V.

Timed event parameters

Number	Event	Units	Range or default	Specify channel
1	Wavelength	nm	190 to 700	Yes
2	Filter time-constant	Seconds	0: Disable filter λ: 0.0125 to 5.00 λλ: 0.5 to 5.0	Yes
3	Sensitivity	AUFS	0.0001 to 4.0000	Yes
4	Chart-mark (10% of full scale)	Does not apply	Does not apply	Yes
5	Polarity	1. + 2. –	+	Yes
6	Auto-zero	Does not apply	Does not apply	Yes
7	Lamp	1. Off 2. On	Off	No
8	Switch 1	1. High 2. Low 3. Pulse 4. Rect wave	High	No

Timed event parameters (Continued)

Number	Event	Units	Range or default	Specify channel
9	Switch 2	1. High 2. Low 3. Pulse 4. Rect wave	High	No
10	Threshold	AU	–4.0000 to 4.0000 or variable, depending on output selection	Yes

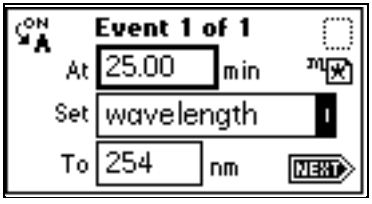
To program a new timed event

1. Press the METHOD (Shift A/B) key on the detector keypad.

   Method choice list

2. Press 1, Timed events.
3. Enter the time for the event.

   Timed events screen

4. Press Enter to enter the time.

   Tip: To advance to the Set field (Events choice list), press the t key.

5. Press Enter again to display the choice list, or, if you know the event number, press the number for the event you are programming.
6. Enter the appropriate wavelength, in nanometers, in the To field, if that field appears.

   Requirement: To program the same event on both channels, you must enter two events, one for channel A and one for channel B.
7. Press A/B to set the threshold on the other channel.

   Tips:

   - ON A or ON B indicates the channel the event is programmed on. You can program all events on channel A, all on channel B, or some on channel A and some on channel B.
   - Event programming is time-based, not channel-specific.
8. Press Next to advance to a new timed event.
9. To delete a timed event, press CE when the time field is active.
10. Press HOME to return to the absorbance screen, and then press Run/Stop.
11. Press Reset.

    Tips:

    - If the detector is under the control of the Waters 717plus Autosampler or another external device, the Inject Start programmed from that device runs the method.
    - If you are working in real time under current conditions (method *), when a power failure or shutdown occurs, you lose all timed or threshold events if you do not store them as a method. (See page 3-39.)

Threshold events

You can program threshold events on channel A and channel B to control the switch contact closure outputs: for example, when using a fraction collector. You can program the switch to change when the programmed output (absorbance, ratio, energy, etc.) on the detector channel A or B is above a

specified threshold. Below the specified threshold, the switch is set as shown in the table.

Threshold events "Set" parameters

Number	Event
1	Set switch 1
2	Set switch 2

Threshold events "To" parameters

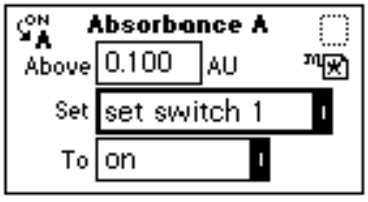
Number	Set to	Below threshold switch state
1	On	Off
2	Off	On
3	Pulse	Off
4	Rect wave (rectangular wave)	Off

To define the pulse period, or the frequency of a wave, see "Configuring the detector" on page 3-22.

To program a threshold event

1. Press the METHOD (Shift A/B) key on the detector keypad.
2. Press 2, Threshold events.

Threshold events screen



3. Press Enter to advance to the next (Set) field, or press the s and t keys to move among the three fields on the threshold events screen.

4. When the Set field is active, press Enter to display the threshold events choice list, or press the number corresponding to the event you are programming (see the table above).
5. When the To field is active, press Enter to display the options in the table shown above, or press the number corresponding to the threshold parameter you are programming.
6. To set the threshold on the other channel, press A/B.

Storing a method

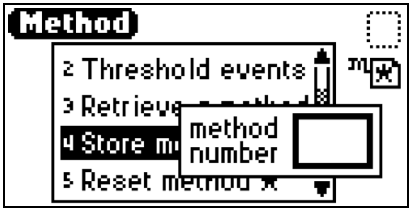
A method consists of all programmable parameters on the absorbance and associated screens as well as timed and threshold events. You can store the current method by selecting a location from 1 to 5.

To store a method

1. Press METHOD (Shift A/B) to return to the Method choice list (see the figure on page 3-36).
2. Press 4, Store method *.

   Result: A method number field appears.

Storing a method, method number field



Caution: No warning message appears when the method number you select is already assigned to a previously stored method. Pressing Enter stores the current method conditions, overwriting any previous method stored in the same slot.

3. Enter a number from 1 to 5 and press Enter.

   Result: A brief message ("Storing * as method *n*") appears.

4. When the display returns to the Method choice list, the method number you selected appears within the method icon. That method remains active until you retrieve another method or reset the detector to default conditions (Method *).

Retrieving a method

To retrieve a previously stored method

1. Return to the Method choice list by pressing METHOD (Shift A/B).
2. Press 3, Retrieve a method.

   Result: The last method number stored or retrieved appears in the method number slot box.

3. Enter the number of the method you want to retrieve, and press Enter.

   Result: A brief message ("Retrieving method *n*") appears.

   When the Method choice list reappears, the method number icon incorporates the method number you specified (see the table on page 3-5).

Viewing events within a method

To view timed and threshold events that make up a stored method

1. Retrieve the method (see page 3-40).

   Result: Once you specify a method number to retrieve, the method choice list appears, and the method number appears within the method number icon.

2. Press 1 to view the timed events or 2 to view threshold events within the displayed method.

   Tip: If you change to a timed or threshold event within a method, the asterisk appears (Method *), indicating that the method (*) is no longer the same as the stored method you retrieved in step 1. You can then store the method containing the altered event(s) in the same storage slot.

Resetting a method

Resetting a stored method is a two-step process. First, you reset the current conditions to the defaults. Then you save the defaults in one of the storage locations. (See page 3-18 for the parameter default settings.)

To clear one or more methods

1. Return to the Method choice list by pressing METHOD (Shift A/B).
2. Press 5, Reset method *.

   Result: A message appears, asking whether you approve of setting current conditions to factory defaults.

   Tip: If you press Enter, these events take place:

   - All timed events are deleted.
   - All threshold events are disabled.
   - All other operating parameters of the method (λ, AUFS, etc.) are set to defaults.

   If you press Cancel (Shift 0), the display returns to the Method choice list.

   Recommendation: To prevent loss of the current conditions, store them in one of the available storage slots before you clear the method. After clearing the storage slots, you can restore the previous conditions.
3. Press 4, Store method, and enter a storage location number.

   Tip: To clear other stored methods, repeat this step until you clear all desired methods.

When you press HOME, the absorbance screen's method number icon displays an asterisk.

Clearing events

You can clear only timed or threshold events without resetting any other operating parameters.

To clear all active timed or threshold events

1. Return to the Method choice list by pressing METHOD (Shift A/B).
2. Press 6, Clear events.

Result: A message appears asking whether you approve of clearing all active events.

Tip: If you press Enter:

- All timed and threshold events in the method are cleared.
- All other operating parameters of the method (λ, AUFS, etc.) are unaffected.

If you press Cancel (Shift 0), the Method choice list appears.

3. When you press HOME, the absorbance screen's method number icon displays an asterisk.

Scanning spectra

The detector must make two scans to produce an absorbance spectrum:

- Zero scan – A reference scan that characterizes the absorbance spectrum of the solvent in the cuvette or the flow cell.
- Sample scan – An absorbance scan of the analyte in solvent (after subtracting out the zero scan of the solvent) to provide the actual spectrum of the sample.

The detector can measure the spectrum of a sample using the cuvette or the flow cell. See page 3-56 and page 3-59 for scanning procedures.

Rule: When using the cuvette, if the contents of the flow cell change, you must rerun the zero scan.

Before you begin

Before you run a spectrum scan, specify values for the following parameters:

- λ1 – Starting wavelength. Scanning begins at this wavelength.
- λ2 – Ending wavelength. Scanning ends at this wavelength.
- Pace – Rate of scanning, in nanometers/min. Determines how fast the scan is output and data are acquired. The scan data are acquired at the

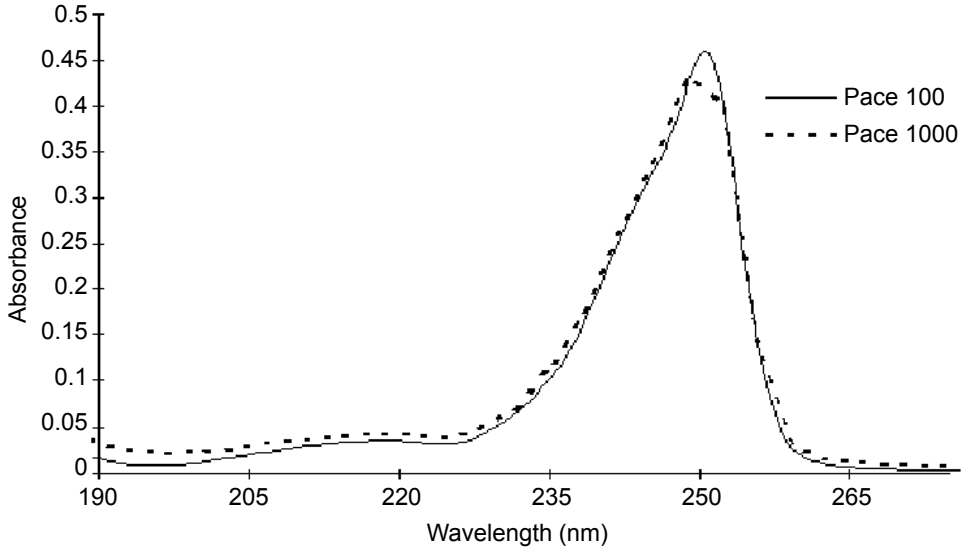
highest possible resolution for the specified pace. Specifying a very high pace reduces resolution.

Pace and sampling resolution examples

Pace (nm/min)	Sampling resolution (nm)
100 and less	0.5
200	1.0
400	2.0

The figure below shows two scans of anthracene, one overlaying the other. At a pace of 1000 nm/min, the overlaid scan (dotted line) shows a reduced number of points scanned, lowering the resolution relative to the original scan, done at a pace of 100 nm/min.

Scan of anthracene at 100 nm/min and 1000 nm/min



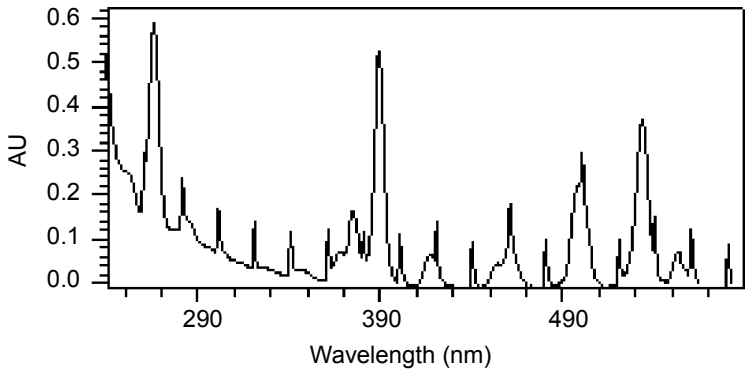
Tip: The higher the number you enter in the Pace field, the lower the resolution of the scan.

- Tick marks – This value allows tick marks (check marks) to be generated at the specified wavelength increment help interpret charted data.

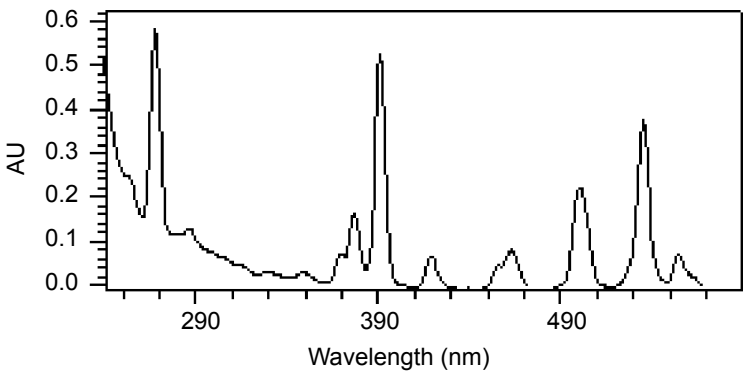
  The figures below show a 190-nm to 600-nm scan of erbium standard in a cuvette at 200 nm/min, with tick marks specified every 20 nm, and the same scan without tick marks.

- AUFS – The sensitivity setting used for scaling the charted spectrum.

Scan of erbium standard in a cuvette from 190 nm to 600 nm at 200 nm/min, with tick marks every 20 nm



Scan of erbium standard in a cuvette from 190 nm to 600 nm at 200 nm/min without tick marks



Specify these parameter values when you select a scan: zero or sample.

When you select a zero scan, the detector displays three additional screens, labeled 2 of 4, 3 of 4, and 4 of 4. You can change all parameters on these screens, including starting and ending wavelength, and the pace parameter.

When you select a sample scan, the detector displays two additional screens, labeled 2 of 3 and 3 of 3 (see the figure on page 3-49). You cannot change the starting and ending wavelength values or the pace parameter.

When you run a zero scan, you establish starting and ending wavelengths, pace, tick marks, and sensitivity for both the zero scan and subsequent sample scans. Run sample scans within 15 minutes of the baseline zero scan.

The most recent zero scan performed or retrieved remains current until you perform or retrieve another scan. The zero scan should be appropriate for the subsequent sample scans you perform. The sample scan adopts the starting and ending wavelengths and pace from the most recent zero scan. Only when those values are identical for both the zero and sample scans can the zero scan be subtracted.

You use the SCAN key (Shift Chart-mark) to run a new zero or sample scan, store, review, subtract and review, and replay stored or existing scans.

During a sample scan, data are charted via analog channel A using the specified AUFS setting. At the same time, sample energy at 150 nA/V is charted via channel B.

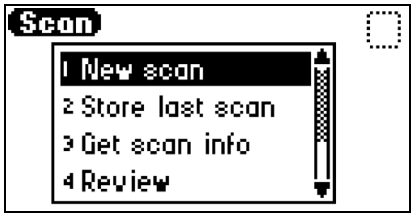
During a zero scan, data are charted via analog channel A. At the same time, reference energy, at 150 nA/V, is charted via channel A, at the AU specified on channel A.

Scanning new spectra

To specify a new spectrum

1. Press SCAN (Shift Chart-mark). A Scan choice list appears.

   #### Scan choice list

2. Press 1, New scan, or use the s and t keys to move through the Scan choice list.

   Result: The detector displays the first of three parameter screens for a sample scan or four parameter screens for a zero scan (figure “Zero and sample scan screen” on page 3-49).

3. Press Next to advance through the New scan parameter screens.

4. On the first New scan screen, specify the type of scan:

   - Press 1 for sample scan, or press Enter to display the choice list. The detector displays two additional screens.
   - Press 2 for zero scan, or press Enter to display the choice list. The detector displays three additional screens.

   All parameters appear on the first New scan screen for both a zero and a sample scan. You can return to screen 1 to review the parameters for either scan type by pressing Next from the Run screen (screen 3 of 3 for sample scan or 4 of 4 for zero scan).

Tip: You can press Run from any New scan screen.

For both zero and sample scans, the table below provides the defaults and ranges for all parameters.

Sample and zero scan parameters

Parameter	Screen	Scan type	Units	Range or default
Type	1	Sample and zero	n/a	Sample scan: 1 Zero scan: 2 Default: 1
λ range	2	Zero scan only	nm	Range: 190 to 700 nm Default: 190 to 700 nm
Pace	2	Zero scan only	nm/min	Range: 30 to 1000 nm/min Default: 100 nm/min
AUFS	2 or 3	Sample and zero	AU	Range: 0.0001 to 4.0000 Default: Last number entered
Tick mark (Mark each nm)	2 or 3	Sample and zero	nm	Range: 10 to 100 Default: Last number entered

Zero scan

To program a zero scan

1. Press SCAN, then 1, New scan, and press 2, Zero scan.
2. Press Next to advance to the second Zero scan parameter screen.
3. Enter the starting wavelength for the zero scan, and then press Enter.
4. Enter the ending wavelength for the zero scan, and then press Enter.
5. Enter a value in the Pace field for the rate at which the detector scans the specified wavelength range.

   Tips:

   - The default is 100 nm/min. The allowable range is from 30 to 1000 nm. Refer to the figure on page 3-43, which shows two scans of anthracene overlaid, one at 100 nm/min and one at 1000 nm/min.
   - The higher the value you enter in the Pace field, the lower the resolution of the scan.

6. Press Next.
7. On the third zero scan parameter screen, enter the AUFS value, and then press Enter.

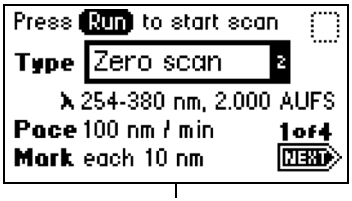
   Tips:

   - To specify tick marks, enter a number from 10 nm to 100 nm, and then press Enter.
   - To clear tick marks, press CE. See page 3-44 for examples of scans made with and without tick marks.

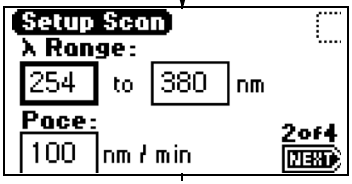
8. Press Run to start the zero scan, or press Next to return to the first zero scan parameter screen to verify parameter values, and then press Run.

   After the detector runs the zero scan, it returns to the Scan choice list.

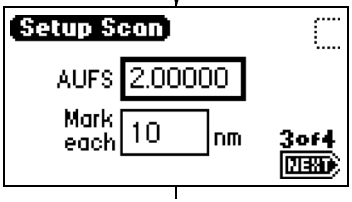
Zero and sample scan screen



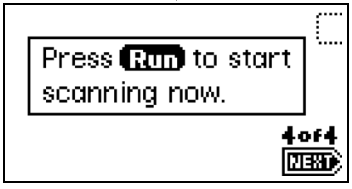
Zero scan (screen 1 of 4)



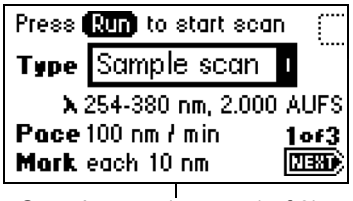
Zero scan (screen 2 of 4)



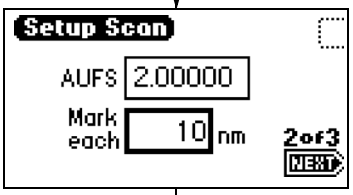
Zero scan (screen 3 of 4)



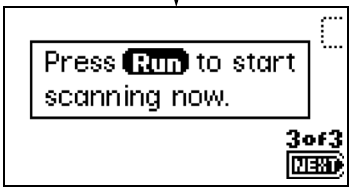
Zero scan (screen 4 of 4)



Sample scan (screen 1 of 3)



Sample scan (screen 2 of 3)



Sample scan (screen 3 of 3)

Running the sample scan

Run the zero scan before running the sample scan. To ensure identical flow cell and solvent conditions, run the sample scan for the corresponding zero scan within 15 minutes of running the zero scan.

To run a sample scan

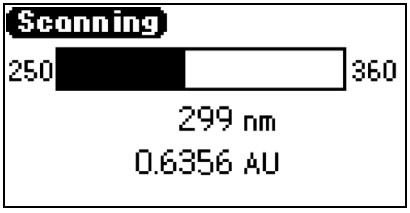
1. Set up the zero (or reference) scan by following the steps in the zero scan procedure on page 3-47.
2. On the first New scan screen, press 1, Sample Scan.

   Result: The parameters for wavelength range, AUFS, Pace, and Mark (tick mark) you entered for the corresponding zero scan appear.
3. Press Next to advance to the second sample scan screen.

   Tip: You can change the entries in the AUFS and Mark each fields.
4. Press Next to advance to the third sample scan screen, and then press Run.

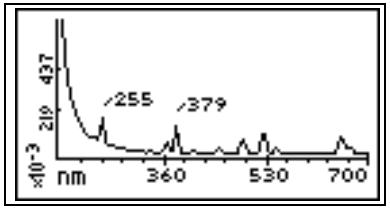
   Result: A brief message ("Initializing") appears. The Scanning screen shows the progress of the scan, in nanometers, across a progress bar.

Scanning progress bar



After a brief pause, the detector displays a sample scan graphically.

Sample erbium scan graphical display



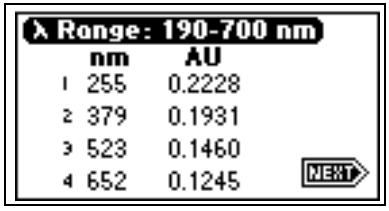
Tip: To return to the Scan choice list, at the end of the scan, press SCAN (Shift Chart-mark).

5. Press Next.

   Result: Doing so displays as many as four of the highest peaks scanned within the specified range.

   The figure below shows the four highest peaks for the erbium scan shown in the graphical display (above).

Sample erbium scan, four highest peaks



6. Press Next.

7. On the graphical display, press Scale (Shift TRACE) to change the scale (zoom in on one part) of the display.

   Tip: You can alter the following four scaling parameters:

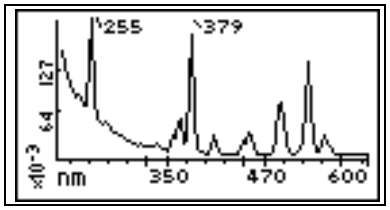
   - λ1 – minimum wavelength displayed.
   - λ2 – maximum wavelength displayed.
   - AU1 – minimum absorbance displayed. The default is Auto.
   - AU2 – maximum absorbance displayed. The default is Auto.

   Using this function, you zoom in on various sections (artifacts) of the spectrum.

   The scale of the spectrum is affected by the AUFS setting.

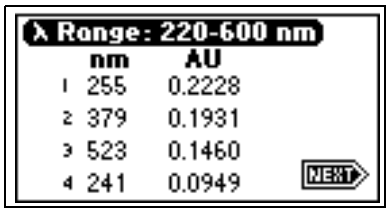
8. Press Next to advance through the four scaling parameters. The figure below shows the sample scanned in the figure on page 3-50. The sample has been scaled by changing the wavelength parameters to 225 and 600 nm.

Sample erbium scan with l1 changed to 225 nm and l2 changed to 600 nm



9. Once you change one or more scaling parameters, press Enter to reformat the graphical display.
10. Once the scan reappears, press Next to display the four highest peaks of the scaled scan.

Four highest peaks from the scaled sample erbium scan

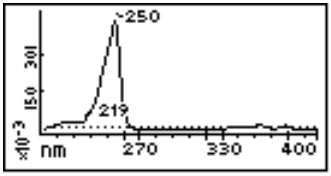


11. Press Next again to return to the sample scan display.
12. When you finish manipulating the sample scan graphical display, press SCAN (Shift Chart-mark) to return to the Scan choice list. To store the scan, see "Storing a spectrum" on page 3-53.

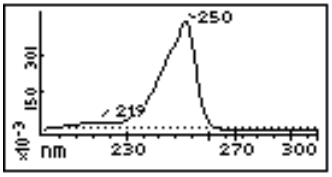
The figure below shows a series of scans of anthracene dissolved in acetonitrile to display the use of the Scale function. The zero scan is not displayed.

For the scaling parameters AU1 and AU2, the default is Auto. You can change the AU parameter based on the absorbance of the spectrum. To return the default to auto, press CE.

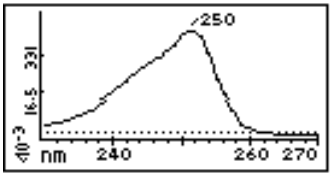
Series of scans of anthracene in acetonitrile



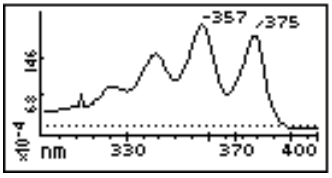
Sample scan
200 nm to 400 nm
–0.001 AU to 0.5 AU
anthracene



Zoom of sample scan
200 nm to 300 nm
–0.001 AU to 0.5 AU
anthracene, 230 nm to 270 nm
λ2 changed to 300 nm



Zoom of sample scan
230 nm to 270 nm
–0.001 AU to 0.5 AU
anthracene, 250 nm
λ1 changed to 230 nm
λ2 changed to 270 nm
AU1 and AU2 on auto



Zoom of sample scan
300 nm to 400 nm
–0.001 AU to 0.025 AU
anthracene, 330 nm to 400 nm
λ1 changed to 300 nm
λ2 changed to 400 nm
AU1 and AU2 on auto

Storing a spectrum

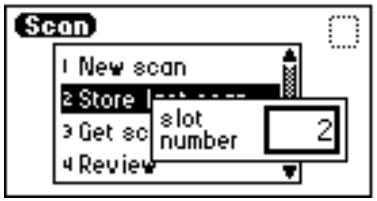
Once you run a spectrum, you can store it for later review, subtraction, or playback. You can store as many as three spectra.

To store a spectrum

1. From the graphic display of a sample scan, return to the first Scan screen by pressing SCAN (Shift Chart-mark).
2. Press 2, Store last scan.

   Tip: When you select Store last scan, you are storing the zero scan and the sample scan as a pair.

   #### Storage slot number box

3. In the slot number box, enter a number from 1 to 3.
4. Press Enter to store the last sample scan, paired with its zero scan.

Getting information about a stored spectrum

To obtain information about a stored spectrum

1. Press SCAN (Shift Chart-mark) to view the Scan choice list.
2. Press 3, Get scan info.

   Result: A slot number box appears with a default of "Last" (the most recently stored spectrum).
3. Press Enter to get information about the last stored spectrum, or type the number (1 to 3) of the stored spectrum for which you want information, and then press Enter.

   Result: A screen containing the following information appears:

   - Storage slot number of the selected scan (or "Last scan")
   - λ range – Displays the wavelength range for the selected spectrum
   - Pace – Displays the pace for the selected spectrum
4. Press Enter to exit the information screen and return to the Scan choice list.

Reviewing a stored spectrum

Once you store a spectrum, you can retrieve it for review from one of the five available storage slots by selecting the Review option from the Scan choice list.

To review a spectrum

1. Press SCAN (Shift Chart-mark) to view the Scan choice list.
2. Press 4, Review.

   Tip: When you select Review, you actually are retrieving the zero scan and the sample scan that were stored as a pair.
3. Enter the storage slot number (1 to 3) of the spectrum you want to review.
4. Press Enter. The message "Retrieving spectrum *n*" appears.

Once you retrieve a spectrum for review, you can view it graphically and adjust the wavelength and AU ranges, if necessary. You can also run a new sample scan based on the retrieved zero scan.

Subtracting a spectrum

Once you store more than one spectrum, you can create a difference spectrum.

Tip: The current spectrum is the spectrum that the stored spectrum (whose slot number you enter) is subtracted from.

To subtract and review the difference spectrum

Rule: To subtract the stored spectrum from the current spectrum, the starting and ending wavelengths ($\lambda 1$ and $\lambda 2$) and the pace of both spectra must be identical.

1. Press SCAN (Shift Chart-mark).
2. Press 5, Subtract & review.
3. Enter the storage slot number (1 to 3) of the spectrum you want to subtract out from the current (or retrieved) spectrum.
4. Press Enter.

Once the difference spectrum appears, you can store the results in one of the three storage slots.

Replaying a spectrum

You can play back the current spectrum or a stored spectrum in real time using the Real-time replay function on the Scan choice list. The detector plays back the selected spectrum in real time both on the detector display and out the analog connector to the chart or A/D device in a data collection system. Once you retrieve a spectrum for playback, the detector displays it graphically, and you can adjust the AUFS. Sample energy is not charted during a replay.

Tip: If you adjust the AUFS on a spectrum for replay, the adjusted spectrum displays only on the charted output, not on the detector graphical display.

To replay a spectrum

1. Press SCAN (Shift Chart-mark).
2. Press 6, Real-time replay.
3. Enter the storage slot number (1 to 3) of the spectrum you want to play back.

   Tip: The default is the last spectrum acquired.
4. Press Enter.

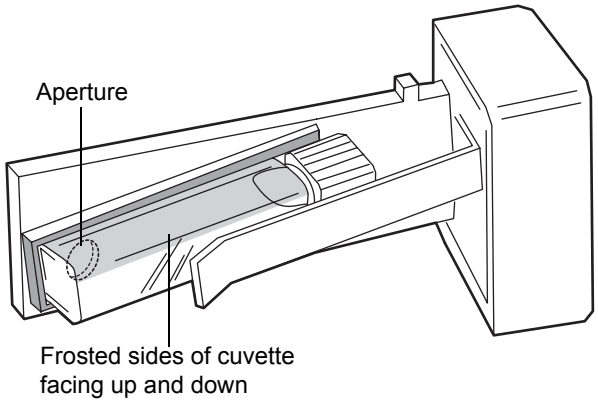
   Result: After a pause, to retrieve the selected spectrum, the detector begins to play back the spectrum on the analog connection. Then the spectrum appears graphically.

Scanning using the cuvette

Using the cuvette option facilitates sample handling and verifying and qualifying the instrument.

The detector uses a standard 10-mm path length spectrophotometric cell (quartz cuvette). You insert the cuvette with one of the two frosted sides facing up in the cuvette holder and place it in the detector flow cell assembly.

Detector cuvette holder with the cuvette inserted



Restriction: Because the scan is actually a composite of both the contents of the cuvette and the flow cell, you need to perform cuvette scans under identical flow cell conditions. If you store spectra and acquire new spectra for subtraction, beware the differences, if any, in flow cell conditions.

Ideally, you perform both the zero and sample scans using the cuvette when the HPLC instruments are in the idle or static state, under identical flow cell conditions.

Caution: Handle the cuvette gently and on the frosted sides only. Fingerprints on the clear quartz interfere with the light path and compromise the integrity of cuvette scanning operations.

Before you begin

Recommendation: To ensure accurate results, use a 10-mm path length quartz cuvette and matched pairs (from the same manufacturing lot) of quartz cuvettes for your zero and sample scans.

Before beginning a scan using the cuvette

1. Flush the flow cell with the eluent you are going to scan.
2. Wipe the clear portion of the cuvette with low-lint, nonabrasive tissue wipes.

Cuvette scanning procedure

To begin a cuvette scan

1. Remove the detector's front, left-hand panel cover.
2. Remove the cuvette holder, sliding it toward you.

Removing the cuvette holder

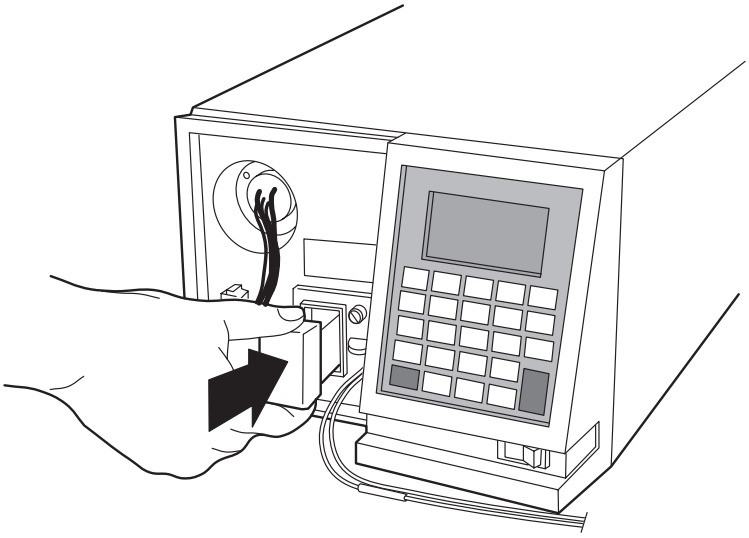
3. With the spring guide facing you, gently insert the cuvette (containing eluent) up under the guide, with the cap facing upward (into the holder) and a frosted side of the cuvette facing up. (See the figure on page 3-57.)

   Recommendations:

   - Ensure that you have enough liquid (3 mL) in the cuvette so that when it is inserted into the holder, you can see liquid through the holder's aperture. That is, the liquid must completely cover the aperture.
   - Because the cuvette holder is angled, use your thumb or forefinger to ensure the cuvette is secure in the slot and does not slide forward. Ensure that it does not become dislodged when you are replacing the cuvette holder.

4. Gently guide the cuvette holder back into the flow cell assembly until it bottoms in the space.

Replacing the cuvette holder



5. Refit the panel.

   Rules:

   - To prevent invalid chromatographic results, remove the cuvette from the detector and replace the empty holder after running cuvette scans.
   - To maintain optimum system performance, refit the front left panel cover before resuming normal operation of the detector.

6. Insert a reference cuvette containing the mobile phase standard, and run a zero scan.
7. Replace the reference cuvette with a cuvette containing your analyte dissolved in mobile phase solvent, and run a sample scan.
8. Use the storage, review, subtract and review, and replay functions to analyze the data obtained.

Scanning using a flow cell and a syringe

If you do not have a cuvette, you can scan using a manually filled flow cell.

Requirement: Before you scan using a flow cell, make sure no cuvette is in the cuvette holder.

To run a spectrum using a flow cell

1. With a syringe, fill the flow cell with the mobile phase or solvent in which your sample is dissolved.
2. Run a zero scan according to the procedure on page 3-46.
3. With a syringe, fill the flow cell with the analyte, and then run a sample scan according to the procedure on page 3-46.

Use the storage, review, subtract and review, and replay functions of the detector to compare the scanned data.

Conserving lamp life

You can conserve the lamp without powering-off the detector by leaving the instrument on and turning off only the deuterium lamp.

Tip: If the detector is operating under remote control, you can program the controller to turn the lamp off and on without using the front panel.

Recommendation: Waters recommends that you program the lamp to shut off or turn the lamp off manually only if the value of the "Lamp off" parameter is more than 4 hours.

Without powering off the system, you can conserve lamp life by

- turning power to the lamp off and on again manually.
- programming a timed event to turn the lamp off and on again.
- programming the lamp to turn off and on again using the external contact closure.

To turn power to the lamp on and off manually, use the Lamp keypad function (Shift 1). When the lamp is turned off, the absorbance screen displays the words "Lamp off", and the lamp icon appears with an "X" through it.

Use the Lamp key (Shift 1) to

- shut the lamp off or turn the lamp on manually.
- display the number of times the lamp has ignited.
- display the hours and minutes the lamp has been turned on during the current run and/or since installation.

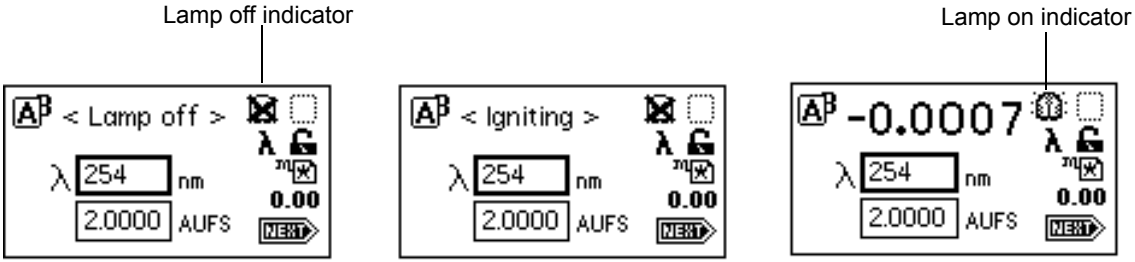
To turn off the lamp manually from the detector front panel

1. Press Lamp (Shift 1) from the keypad. The lamp control screen appears.

Lamp control screen

2. Press Lamp (Shift 1) again to turn the lamp off.

Lamp off/on sequence



To ignite the lamp manually

1. When there is an X through the lamp icon on the absorbance screen, press Lamp (Shift 1).
2. Press Lamp (Shift 1) again to turn the lamp on. The absorbance screen appears with the message “Igniting.”

   Result: The lamp can take as many as 1 minute to illuminate. Once the lamp illuminates, the display returns to the absorbance screen, and the X is removed from the lamp icon.

You can conserve lamp life by programatically igniting and extinguishing it: for example, overnight, using a timed event method.

To ignite or extinguish the lamp programatically, select the Timed events option in the Method choice list, or program the lamp through one of the external contact closures.

- See “Programming timed events, threshold events, and methods” on page 3-34, and the table on page 3-35 for more information on programming the lamp to turn on or off using a timed event.
- See “Configuring event inputs (contact closures)” on page 3-23 for more information on programming the lamp through the external contact closure.

Shutting down the detector

If you need to power-off the detector for an extended length of time, you must remove any buffered mobile phase from the fluid path.

Caution: To avoid damage to the column, remove it before you perform the following procedure. Before you remove the column, see your column care and use guide.

Removing buffered mobile phase

To remove mobile phase from the fluid path of the detector

1. Replace the buffered mobile phase with 100% HPLC-quality water and flush the system for 10 minutes at 3 mL/min.
2. Replace the 100% water mobile phase with a solution of 90:10 methanol/water, and flush the system for 10 minutes at 3 mL/min.

Follow the recommended procedures for injector purging and priming for the pump in use with your HPLC.

Shutting down the detector

To shut down the detector, press the On/Off switch.

4 Maintaining the Detector

Contents:

Topic	Page
Contacting Waters technical service	4-2
Maintenance considerations	4-2
Proper operating procedures	4-4
Maintaining the flow cell	4-5
Replacing the lamp	4-16
Replacing fuses	4-25

Contacting Waters technical service

If you are located in the USA or Canada, report malfunctions or other problems to Waters Technical Service (800 252-4752). Otherwise, phone the Waters corporate headquarters in Milford, Massachusetts (USA), or contact your local Waters subsidiary. Our Web site includes phone numbers and e-mail addresses for Waters locations worldwide. Go to www.waters.com.

When you contact Waters, be prepared to provide this information:

- Completed normal operation checklist for the method you are using
- Nature of the symptom
- Instrument serial number
- Flow rate
- Operating pressure
- Mobile phase(s)
- Detector settings
- Type and serial number of column(s)
- Sample type
- Control mode (Empower, MassLynx, FractionLynx™, No interaction, or other)
- Software version and serial number

For complete information on reporting shipping damages and submitting claims, see *Waters Licenses, Warranties, and Support Services.*

Maintenance considerations

Perform the procedures in this chapter when you discover a problem with a 2489 detector component or during preventive maintenance.

Safety and handling

Observe these warning and caution advisories when you perform maintenance operations on your 2489 detector.

Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the 2489 detector. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.

Warning: Avoid electric shock:

- Do not open the detector's cover. The components within are not user-serviceable.
- Power-off and unplug the detector before performing any maintenance operation on the instrument.

Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the detector. To completely interrupt power, set the module's power switch to "off", and then unplug the power cord from the AC outlet. Wait 10 seconds before you disconnect an assembly.

Spare parts

Replace only parts mentioned in this document. For spare parts details, see the Waters Quality Parts Locator on the Waters Web site's Services & Support page.

Removing the front left panel cover

Caution:

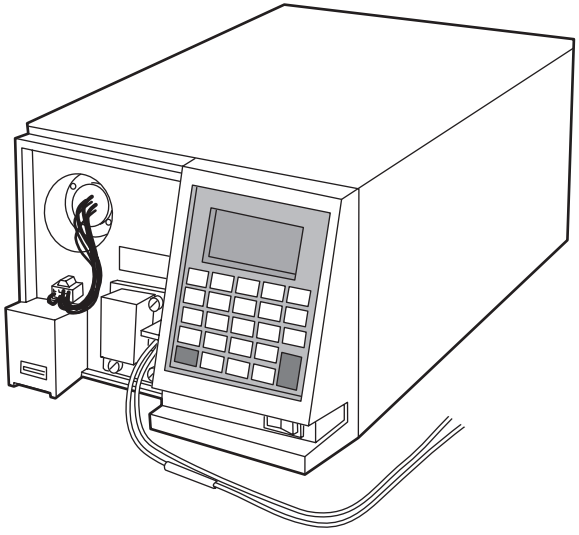
- There are no user-serviceable parts inside the detector. Do not remove the top cover.
- To maintain optimum performance, replace the front left panel cover before resuming normal operation of the detector.

To remove the front left panel cover

1. Holding on to the top of the cover, gently pull the bottom away from the unit.
2. Gently remove the top of the cover, and store it nearby.

The following figure illustrates the detector with the front, left-hand panel cover removed.

Detector with front left front panel cover removed



Routine maintenance

The detector requires minimal routine maintenance.

To achieve optimal performance,

- replace solvent reservoir filters in your HPLC system regularly.
- filter and degas solvents to prolong column life, reduce pressure fluctuations, and decrease baseline noise.
- flush buffered mobile phases out of the detector with HPLC-grade water followed by a 5 to 10% methanol solution each time the detector is powered-off. This process prevents
  - plugging of the solvent lines and flow cell.
  - damage to the components.
  - microbial growth.

Maintaining the flow cell

A dirty flow cell can cause baseline noise, decreased sample energy levels, calibration failure, and other problems with operation of the detector.

There are two phases in cleaning the flow cell:

- Flushing
- Removing and cleaning

If flushing is ineffective, remove and clean the flow cell. Replace flow cell components as required.

Caution: Always replace the flow cell gaskets when cleaning, rebuilding, or replacing other flow cell components.

This section provides information about the following procedures:

- Flushing the flow cell
- Removing and cleaning the flow cell
- Disassembling and reassembling the flow cell

Flushing the flow cell

Flush the flow cell when it becomes contaminated with the residues of previous runs and after each detector shutdown. A dirty flow cell can cause baseline noise, decreased energy levels, calibration failure, and other problems. Always flush and purge the flow cell as your initial attempt to correct these problems.

You should flush the flow cell whenever

- noise is higher than expected.
- Noise test results are not meeting specifications
- the detector fails to normalize.

Caution: To avoid damaging the flow cell during reverse flushing, do not overpressure the cell.

If you use buffered mobile phase, flush it from the detector before powering-off.

Caution:

- If the flow cell will not be used for several days, flush it with the clean mobile phase, such as a water/acetonitrile or water/methanol, then cap the flow ports or dry the flow cell with pure nitrogen or pure helium.
- To prevent flow cell failure, do not connect any tubing or device that can create backpressure exceeding the flow cell's maximum rating of 3447 kPa (34 bar, 500 psi).

Requirement: Always use well-degassed eluents.

To flush the flow cell

1. Stop the solvent flow, and remove the column.
2. Replace the column with a union or piece of tubing.

   Caution: If the mobile phase is not compatible with water, flush with an intermediate solvent first.

3. Flush the detector with HPLC-quality water.

4. Pump 100% methanol through the flow cell to clean it internally. Do not exceed 3447 kPa (34 bar, 500 psi).
5. Pump a strong cleaning solvent, such as isopropanol, through the flow cell (optional). Do not exceed 3447 kPa (34 bar, 500 psi).

   Caution: If the mobile phase is not miscible in water, first use an intermediary solvent.

6. Resume pumping mobile phase.
7. Reattach the column.

   Tip: Waters recommends renormalizing in 100% water before resuming analyses.

8. If the flow cell remains dirty or blocked, reverse flush it.

Removing and cleaning the flow cell

If flushing the flow cell is not effective, follow the procedures to remove the flow cell and inspect for dirty or broken windows or dirty gaskets. Clean and replace parts if necessary.

Before removing the flow cell assembly, purge the flow cell with nitrogen, and allow it to dry.

To purge the flow cell

1. Connect the nitrogen supply line to the sample inlet. Route the sample line to waste.
2. Purge the cell for 25 to 30 minutes at 103 to 138 kPa (1 to 1.4 bar, 15 to 20 psi).
3. Allow the flow cell to dry completely.
4. Disconnect the detector inlet/outlet tubing from the main column connection and cap.

Disassembling and reassembling the flow cell

Before you begin

Take the following precautions when disassembling and reassembling the flow cell:

- To prevent contamination, use powder-free finger cots or gloves when touching the flow cell lenses or window.
- Take care to prevent scratching of the flow cell parts.
- Use a clean nonparticulating cloth or a similar clean surface in the work area where you are removing, rebuilding, and replacing the flow cell.

Caution: Always replace the flow cell gaskets when cleaning, rebuilding, or replacing other flow cell components.

Required materials

You need the following items to remove, clean, and replace the flow cell:

- 1/4-inch flat-blade screwdriver
- Ethyl alcohol or methanol
- Flow cell rebuild kit
- Lint-free swab
- Nitrogen
- Plastic tweezers
- Powder-free finger cots or gloves
- Torque wrench set to 0.904 N•m (128 in-oz, or 8 in-lb) with 1/4-inch flat-blade screwdriver bit

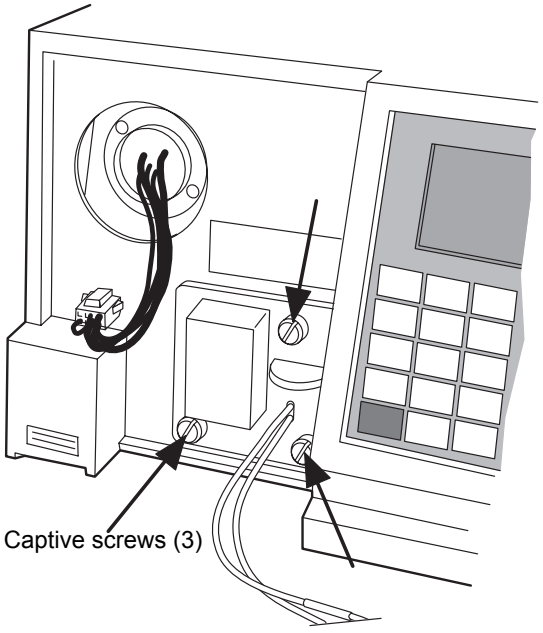
Save all parts after removal unless otherwise indicated. Most of the removed parts are needed when you reinstall a flow cell.

Removing the flow cell assembly

To remove the flow cell assembly

1. Power-off the detector and disconnect the power cord.
2. After flushing and drying the flow cell (see page 4-6), disconnect and cap the inlet and outlet LC tubing to the detector.
3. Remove the front, left-hand panel cover.
4. Using a 1/4-inch flat-blade screwdriver, loosen the three captive screws on the flow cell assembly front plate.

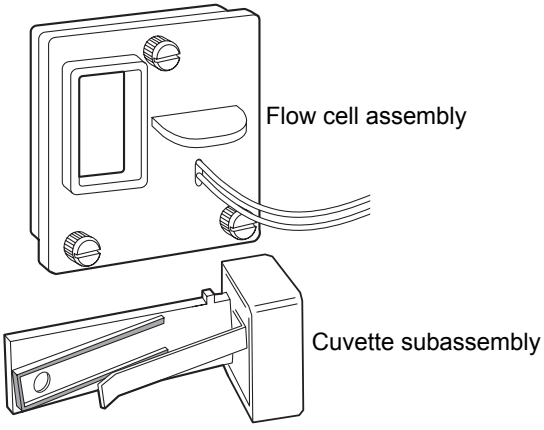
Flow cell assembly captive screws



5. Gently pull the assembly toward you.

6. Once you remove the flow cell assembly from the detector chassis, remove the cuvette subassembly from the flow cell assembly.

Detector flow cell assembly with the cuvette subassembly removed



7. Place the flow cell assembly on a flat clean surface.

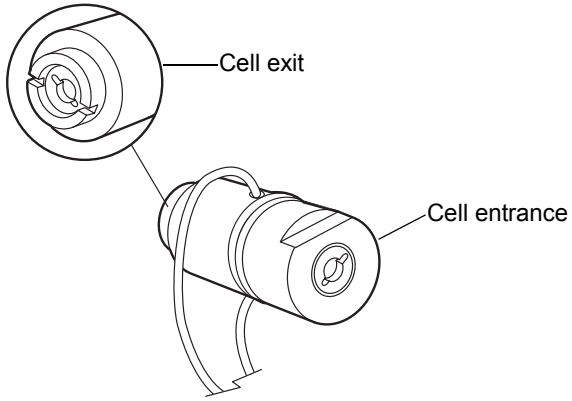
Disassembling the flow cell

Caution: To prevent contamination, use powder-free finger cots or gloves when disassembling, inspecting, cleaning, or replacing parts within the Waters TaperSlit™ Flow Cell or when removing or replacing the flow cell within its assembly.

The TaperSlit flow cell consists of these components:

- Flow cell body
- Cuvette lens
- Split ring (cuvette lens holder)
- Cuvette lens screw
- Lens mount screw
- Exit window
- Exit window mount
- Entrance lens holder
- Entrance lens
- Entrance lens mount
- Two gaskets

Waters TaperSlit flow cell



For replacement parts for the TaperSlit flow cell, use the Flow Cell Rebuild Kit.

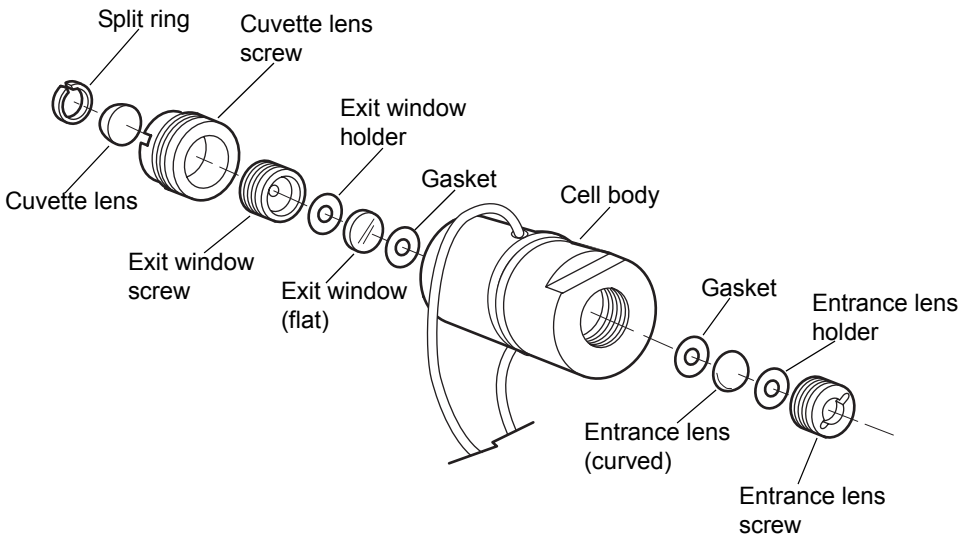
Tip: Use nitrogen to clean the flow cell. Use ethanol or methanol to clean the lenses and window.

To remove the individual parts of the flow cell for cleaning or replacement

1. With the notches on the flow cell cuvette lens end facing you, use the flat-blade screwdriver or a coin to remove the cuvette lens screw.
2. Insert the cotton end of the lint-free swab into the cuvette end of the flow cell assembly, and remove the split ring and the cuvette lens.
3. Using the screwdriver, remove the exit lens mount screw.
4. Using plastic tweezers, pull out the exit window from inside the exit lens mount screw.
5. Using the plastic tweezers or the lint-free swab, remove the clear plastic gasket.
6. Flip the flow cell over to the entrance lens side.
7. Using the flat-blade screwdriver, remove the entrance lens screw.
8. Using the plastic tweezers, remove the entrance lens from inside the entrance lens screw.
9. Using the plastic tweezers or the lint-free swab, tap and remove the clear plastic gasket.

The following figure shows an exploded view of all parts of the flow cell within the flow cell assembly.

Waters TaperSlit flow cell, exploded view



Inspecting, cleaning, and replacing damaged flow cell components

Caution: To prevent contamination, use powder-free finger cots or gloves when disassembling, inspecting, cleaning, or replacing parts within the Waters TaperSlit Flow Cell or when removing or replacing the flow cell within its assembly. Work on a clean flat surface, such as a nonparticulating cloth or similar surface.

To inspect or clean the parts of the flow cell or to replace damaged parts of the flow cell such as the lenses, exit window, or gaskets, follow this procedure and the procedure on page 4-14.

Recommendation: Replace the clear plastic gaskets each time you inspect and clean the flow cell.

To inspect and clean the flow cell

1. Inspect each part of the removed flow cell for dirt.
2. Using ethanol or methanol, clean the contaminated part. Blow dry with nitrogen.
3. Using the Flow Cell Rebuild Kit, replace any flow cell parts that are scratched, burred, damaged, or do not come clean using nitrogen.
4. Rebuild the flow cell by following the procedure in the next section.

Rebuilding the flow cell

After cleaning or replacing other flow cell parts, rebuild the flow cell.

To rebuild the flow cell

1. Using the plastic tweezers, remove the new clear plastic gaskets from the Flow Cell Rebuild Kit, and inspect them for dirt.
2. Drop one clear, plastic gasket into the groove at the bottom of the entrance lens end of the flow cell body.
3. Inspect the entrance lens, and use nitrogen to blow dust off, if necessary.
4. Using plastic tweezers, drop the entrance lens into the flow cell body, curved-side facing up.
5. With the tan-colored entrance lens holder curve facing down, use the torque wrench to tighten the entrance lens screw into the flow cell body to 0.904 N•m (128 in-oz, or 8 in-lb).
6. Flip the flow cell body over to the exit window side.
7. Using plastic tweezers, inspect the second new gasket for cleanliness.
8. Drop the clear plastic gasket into the groove at the bottom of the cuvette lens end of the flow cell body.
9. Inspect the exit window for cleanliness. Using nitrogen, clean the exit window, if necessary.
10. Using plastic tweezers, place the exit window into the flow cell body.
11. With the tan-colored exit window holder facing down, use the torque wrench to tighten the exit window screw into the flow cell body to 0.904 N•m (128 in-oz, or 8 in-lb).

Tip: To ensure complete compression of the gaskets, after replacing the exit window and torquing the exit window screw, and after replacing the cuvette lens and torquing the cuvette lens screw, you must flip the flow cell body and torque the entrance lens screw to 0.904 N•m (128 in-oz, or 8 in-lb) a second time.

12. Flip the flow cell body again, and torque the entrance lens screw to 0.904 N•m (128 in-oz, or 8 in-lb).
13. Flip the flow cell body again to the cuvette lens end, and torque the exit lens screw again to 0.904 N•m (128 in-oz, or 8 in-lb).
14. With the curved side of the cuvette lens facing up, replace the lens in its screw mount.
15. With gloved fingers, place the split ring onto the cuvette lens, and press it firmly until it is flush on all sides.
16. Using the cuvette tool, turn the cuvette lens screw into the exit-lens-mount end of the flow cell body.
17. Torque the cuvette lens screw to 0.904 N•m (128 in-oz, or 8 in-lb).
18. Follow the procedures in the next section to refit the flow cell in the flow cell assembly.

Replacing the flow cell

Caution: To prevent contamination, use powder-free finger cots or gloves when disassembling, inspecting, cleaning, or replacing parts within the Waters TaperSlit Flow Cell or when removing or replacing the flow cell within its assembly.

The detector is shipped with a standard analytical flow cell installed. Replace the flow cell when

- the flow cell becomes damaged.
- you want to use one of the optional flow cells (see the table on page B-5).

To prepare for replacing the flow cell

1. Unpack and inspect the new flow cell.
2. Power-off the detector and disconnect the power cord.
3. Remove the front, left-hand panel cover.

4. Disconnect the detector inlet/outlet tubing from the main column connection and cap.

To replace the flow cell

1. Using a 1/4-inch flat-blade screwdriver, loosen the three captive screws on the front plate of the flow cell assembly (see the figure on page 4-9).
2. Gently pull the assembly toward you.
3. Insert the new flow cell assembly into the detector.
4. Tighten the captive screws.
5. Reconnect the inlet/outlet tubing to your LC system.
6. Reconnect the power cord, and then power-on the detector.

Replacing the lamp

Recommendation: Waters recommends that you change the detector lamp when it repeatedly fails to ignite, or when the detector fails to calibrate.

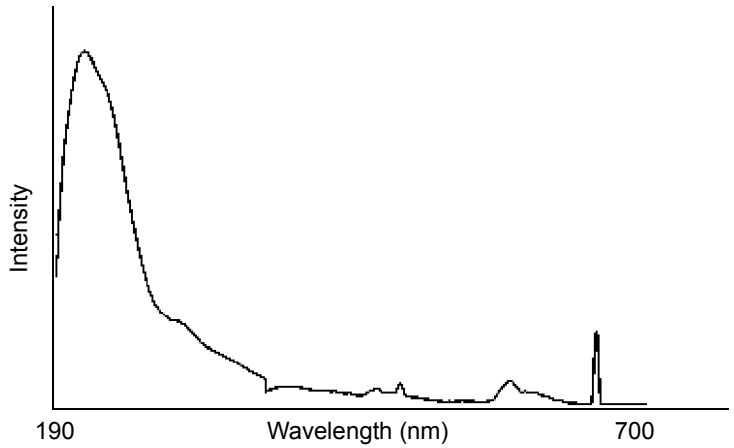
See also: The tables titled “Start up, calibration and operating error messages” (page 5-3), “Instruments error messages” (page 5-6), and “Detector diagnostic tests” (page 5-10).

This section describes the procedure for removing and replacing the detector deuterium lamp.

Lamp characteristics

The intensity of the deuterium source lamp varies by wavelength as shown in the figure below.

Deuterium lamp sample beam intensity profile



Lamp energy and performance

As the lamps used in traditional detectors age, the signal-to-noise performance of the instrument degrades. Determining the longevity of useful lamp life can be difficult because of varying user requirements and individual lamp behavior.

The detector's design also compensates for the lamp energy changes that occur across the deuterium spectrum as well as lamp aging. Thus the detector can operate at the same, high signal-to-noise performance at longer wavelengths of the visible range without the use of a second lamp, such as tungsten.

The detector performs several self-diagnostic tests when powered-on or when the Calibrate key is selected. One of these tests is the lamp optimization software routine. After the detector verifies the calibration of the monochromator, it evaluates energy levels in several characteristic regions across the spectrum. The integration time of the front-end electronics is adjusted to maximize the signal within these regions. The effect is to maintain a high signal-to-noise ratio and operate with a clean signal.

When operating the detector, run the lamp optimization software routine at least once a week.

Eventually, the lamp's signal becomes low enough that replacement becomes necessary. You should replace the lamp when the energy value approaches 15 nanoamps (nA), which corresponds to the cutoff adopted in the detector's diagnostic tests. Ultimately, however, the detector's performance is a function

of its application so replace lamps whenever you determine doing so is appropriate for your method.

The detector's on-board diagnostic tests allow the recording of lamp usage and reporting of the lamp serial number.

When to replace the lamp

Rule: Always run the Change Lamp diagnostic test after you install a new lamp (see page 5-15).

Replace the lamp when

- it fails to ignite at startup.
- the energy level in the lamp causes a decrease in sensitivity to the point where the baseline is too noisy for your LC application.

Rule: Always run the procedure in "Recording sample and reference beam energies" on page 3-28 when you change the detector lamp.

Waters warrants the lamp to ignite and pass startup diagnostic tests for 2000 hours or one year from the date of purchase, whichever comes first.

Removing the lamp

Warning: The lamp housing gets extremely hot during operation. To prevent burn injuries,

- allow the lamp to cool for 30 minutes before removing it.
- keep the lamp in the housing when handling the lamp.

Warning: To avoid eye injury from ultraviolet radiation exposure,

- power-off the detector before changing the lamp.
- wear eye protection that filters ultraviolet light.
- keep the lamp in the housing during operation.

To remove the lamp

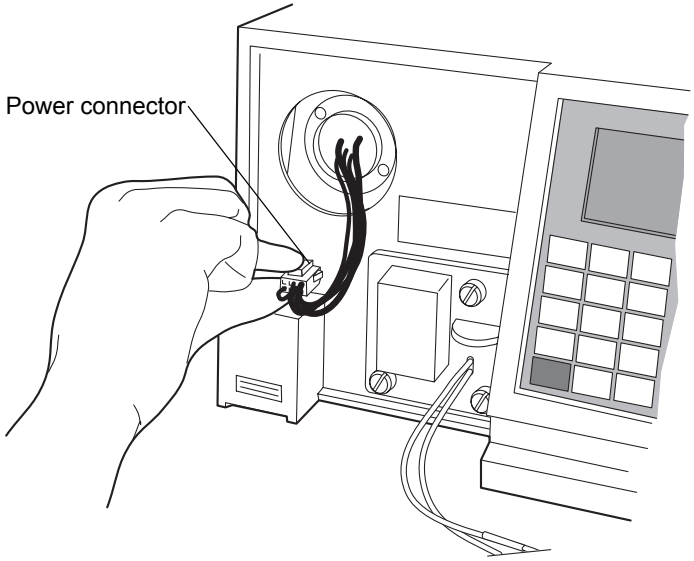
1. Extinguish the lamp using the keypad by pressing <Shift> <Lamp> and then pressing <Shift> <Lamp> again.

   Tip: Using the keypad to extinguish the lamp allows the lamp to cool faster because of the operation of the instrument's cooling fan. To

power-off the lamp using a timed event, see the instructions in the Empower or MassLynx Help.

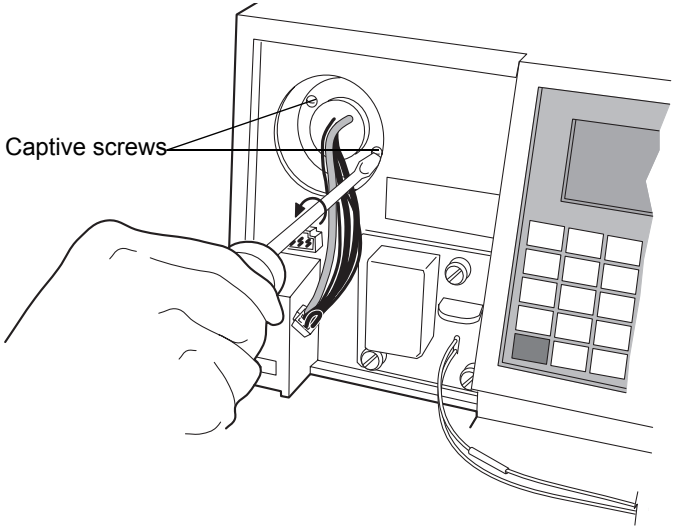
2. Power-off the detector, and disconnect the power cord.
3. After extinguishing the lamp, allow it to cool for at least 30 minutes.
4. Remove the front, left-hand panel cover, as described in "Proper operating procedures" on page 4-4.
5. Disconnect power to the lamp and disconnect the cord.

Lamp assembly and power connector



6. Loosen the two captive screws in the lamp base.

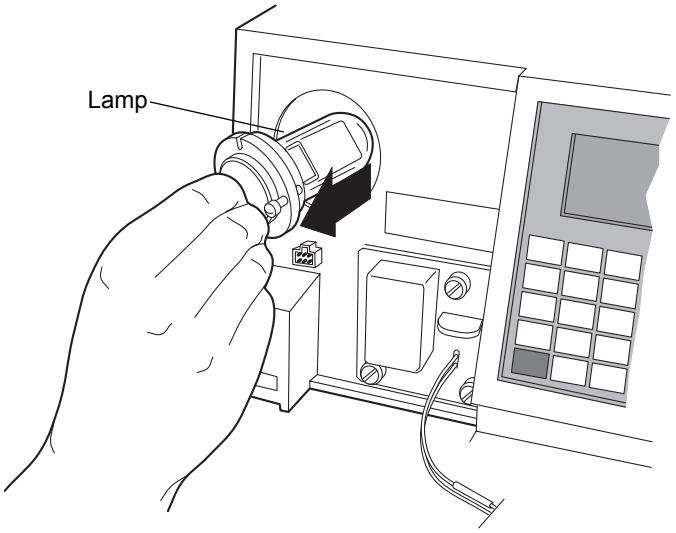
Loosening the captive screws at the lamp housing base



7. Withdraw the lamp assembly from the lamp housing.

Caution: Lamp gas is under slight negative pressure. To prevent shattering the glass, use care when disposing of the lamp. Cushion the old lamp by placing it in the packaging material of the new lamp before disposal.

Removing the lamp



Installing the new lamp

Warning: To avoid exposing your eyes to harmful ultraviolet radiation, never ignite a lamp while it is outside the instrument or if it is not properly secured in place.

Caution: Do not touch the glass bulb of the new lamp. Dirt or fingerprints on the bulb adversely affect detector operation. If the lamp needs cleaning, gently clean the bulb with ethanol and lens tissue. Do not use abrasive tissue or apply excessive pressure.

Before you begin

1. Unpack the lamp from its packing material.

   Note: The new lamp's features can vary slightly from those shown in the figure.

2. Inspect the new lamp for particles or dirt. If necessary, clean the lamp with a gas duster or lens tissue.

3. Record the serial number located on a label attached to the lamp connector wire using the procedure in "Recording the new lamp serial number" on page 4-23.

Requirement: Make sure the detector is powered-off and the power cord is disconnected.

To install the new lamp

Caution: When you change the lamp, always power-off the detector. After installing a new lamp, power-on the detector and always wait at least 5 minutes for the new lamp to warm up.

1. Position the lamp so the cut-out located on the lamp base is at the 1 o'clock position (see the figure below), in line with the alignment pin on the lamp housing. There is no additional alignment required.

2. Gently push the lamp forward until it bottoms into position.

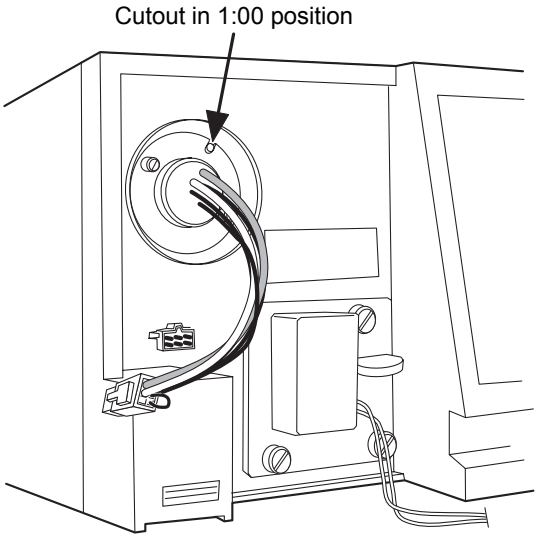
3. Tighten the two captive screws.

4. Reconnect the lamp's power connector.

5. When ready to resume operation of the detector, reconnect the power cord and power-on the unit.

   Tip: The instrument firmware automatically delays operation for 5 minutes to allow the lamp to warm after it has been reignited.

Aligning the lamp



Recording the new lamp serial number

Caution:

- Always run the Change Lamp diagnostic test after you install a new lamp (see "Using the lamp, display, and keypad diagnostic tests" on page 5-15).
- If you do not record the new lamp serial number following the procedure in this section, the lamp warranty is voided.

The detector software records and stores the serial number and date of installation of a new lamp so that you can periodically check its age and its number of ignitions.

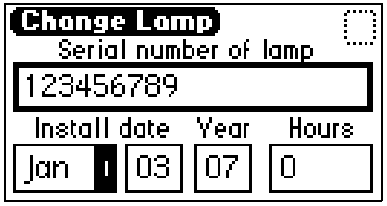
To record the new lamp serial number

1. When the detector warms up, press the DIAG key.
2. Press 4, Lamp, display & keypad.
3. Press 1, Change lamp.

   Tip: Be sure to enter the 9-digit lamp serial number and not the lamp part number when performing this procedure.

4. Enter the 9-digit serial number of the new lamp in the active field.

Change lamp screen



5. Press Enter to store the serial number and to move to the "Install date" field.
6. Select the month from the choice list, and press Enter twice to update the month and to select the next field, which specified the day.
7. Specify the day of the month the lamp is installed, and then press Enter to enter it and move to the next field, which specifies the year.
8. Specify the year (last two digits only), and press Enter to update the year and to select the Hours field.

   Tip: The Hours field is optional. If you are using a lamp for which hours of use are already recorded, enter those hours of use. If this is a new lamp, enter 0 hours are 0.
9. Press the HOME key.
10. At the "OK to store" message, press Enter to store the serial number and date of installation, or press Cancel to cancel the entry.
11. When the confirmation message appears, press Enter.
12. Perform a manual wavelength calibration (see page 3-30).

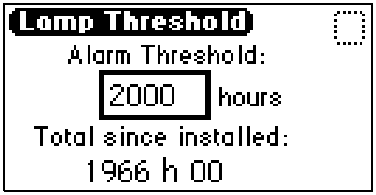
Requirement: To run the verification procedures with the new lamp installed, recalibrate the detector, or cycle power to the detector after replacing the lamp.

Setting the lamp threshold

You can set an alarm threshold for the lamp. When the number of hours meets or surpasses the threshold, an alarm message appears. The default alarm threshold is 2000 hours.

The alarm message also appears when you first power-on the instrument. The lamp threshold screen displays the total hours of lamp use since the lamp's installation.

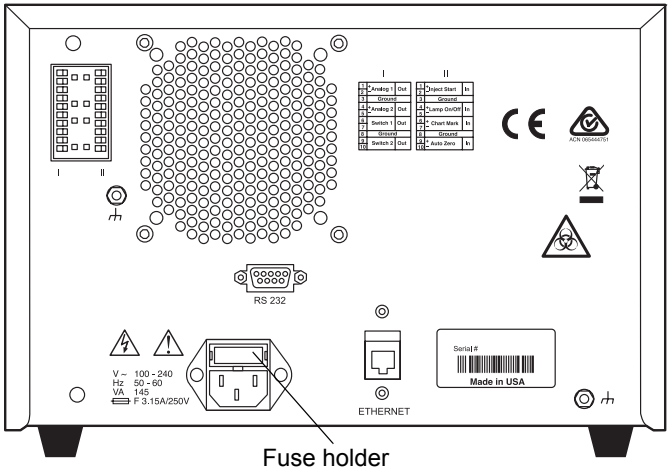
Lamp alarm threshold screen



Replacing fuses

The fuse holder is located on the rear panel of the detector (see figure below). Two fuses are shipped in place with the detector, rated as indicated in "Power requirements" on page 2-4.

Detector rear panel fuse holder



Warning: To avoid electric shock, power-off and unplug the instrument before inspecting the fuses.

The detector requires two 100 to 240-VAC, 50 to 60-Hz, F 3.15-A, 250-V fast blo, 5 × 20 mm (IEC) fuses.

Suspect a fuse is open or otherwise defective when

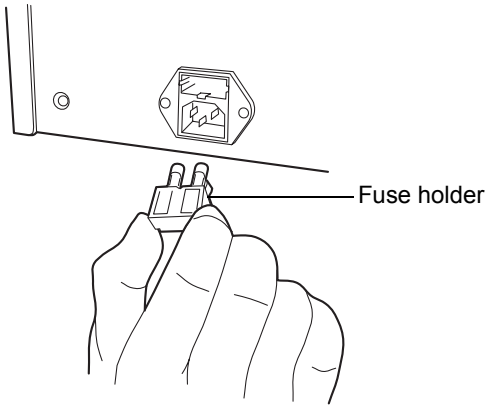
- the detector fails to power-on.
- the fans do not operate.

To replace the fuses

Requirement: Replace both fuses, even when only one is open or otherwise defective.

1. Power-off the detector and disconnect the power cord from the power entry module.
2. Pinch the sides of the spring-loaded fuse holder, which is above the power entry module on the rear panel of the detector.
3. With minimum pressure, withdraw the spring-loaded fuse holder.

Removing and replacing the rear panel fuses and fuse holder



4. Remove and discard both fuses.
5. Make sure that the new fuses are properly rated, and then insert them into the holder and the holder into the power entry module, gently pushing until the assembly locks into position.
6. Reconnect the power cord to the power entry module.

5 Error Messages, Diagnostic Tests, and Troubleshooting

The detector provides both user and service diagnostic tests to troubleshoot system problems.

Contents:

Topic	Page
Error messages	5-2
User-selected diagnostic tests	5-9
Troubleshooting	5-20

Error messages

Startup error messages

Startup diagnostic tests run automatically when you power-on the detector. They verify the proper operation of the detector electronics. If one or more of the tests fail, the detector beeps and displays an error message. For serious errors, it displays the word “Error” in brackets (<Error>) in place of the run-time absorbance on the absorbance screen.

Tip: To reduce the likelihood of errors, be sure the cuvette holder is empty, the flow cell contains degassed transparent solvent (methanol or water), and the front, left-hand panel cover is attached securely.

The tables in this section are organized as follows:

- Messages requiring you to perform corrective action including messages encountered at startup and during calibration or operation.
- Messages requiring you to cycle power, and then contact Waters Technical Service personnel if an error persists (see “Contacting Waters technical service” on page 4-2). Most of these errors arise on startup.

The table below provides startup, calibration, and operating error messages, descriptions, and recommended actions you can take to correct the problem.

Startup, calibration, and operating error messages

Error Message	Description	Corrective Action
Calibration differs: *n* nm	At startup, the unit performs a complete verification, which includes remeasurement of all calibration points. New calibration points are compared to stored information from the most recent manual calibration. If any one of the points differs by more than 1.0 nm, the detector displays this message.	1. Cycle power to the detector. 2. Perform manual calibration. 3. Contact Waters Technical Service.
Calibration not found	Stored calibration data not valid.	Perform manual calibration procedure.
Calibration unsuccessful: Peak out of range *n.nn* nm	Results of a calibration operation differ by more than 1.0 nm. Unit uses previously stored calibration points.	1. Remove cuvette. 2. Be sure the front left panel door is closed. 3. Flush flow cell.
Lamp external input conflict	A timed event or action from the unit's front panel attempts to change the lamp state in conflict with enabled lamp input contact closure.	1. Check contact closure status. 2. Check timed events. 3. Cycle power to the detector.

Startup, calibration, and operating error messages (Continued)

Error Message	Description	Corrective Action
Lamp failure	Lamp indicates Off when it should be On.	1. Check lamp icon. 2. Cycle power to the detector. 3. Replace lamp.
Lamp lighting failure	The lamp failed to ignite.	1. Cycle power to the detector. 2. Check lamp power connection. 3. Replace lamp.
Peak not found: Erbium *n* nm	Erbium filter calibration range does not contain a local maximum.	1. Remove cuvette. 2. Be sure the front left panel door is closed. 3. Flush flow cell.
Peak not found: 656 nm deuterium	Unit sensors cannot determine 656-nm peak.	1. Remove cuvette. 2. Be sure the front left panel door is closed. 3. Flush flow cell.

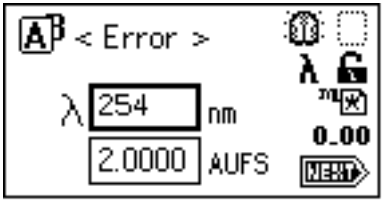
Startup, calibration, and operating error messages (Continued)

Error Message	Description	Corrective Action
Wavelengths span 370 nm: Order filter not in use	In dual-wavelength mode: • If both selected wavelengths > 370 nm, the detector applies the second-order filter to block unwanted UV light. • If both selected wavelengths < 370 nm, the detector removes the second-order filter. • If the selected wavelengths bracket the 370-nm threshold, the detector does not apply the second-order filter and issues a warning message that any data collected for wavelengths above 370 nm may contain inaccuracies because of possible UV light interference (second-order effects).	Select wavelengths that are each above or below 370 nm.

Error messages preventing operation

During initialization, calibration, and operation, the detector can display "<Error>" on the absorbance screen, signifying a usually terminating malfunction and preventing further operation of the detector.

Error display on the absorbance screen



When you encounter such an error, ensure that

- no cuvette is in the cuvette holder, and the empty holder is securely in place.
- the flow cell is clean.
- the front, left-hand panel door is shut securely.

Cycle power to the detector. If the terminating error persists, contact Waters Technical Service.

Instrument error messages

Error Message	Description	Corrective Action
Communication failure: Reference A/D	A/D communication test failed.	1. Cycle power to the detector. 2. Contact Waters Technical Service.
Communication failure: Sample A/D	A/D communication test failed.	1. Cycle power to the detector. 2. Contact Waters Technical Service.
Configuration not found	Stored configuration data are invalid.	Cycle power to the 2489 Detector. This action removes the error.
Dark current too high: *nnnnnnn*	The dark energy level is above 1000000.	1. Cycle power to the detector. 2. Contact Waters Technical Service.

Instrument error messages (Continued)

Error Message	Description	Corrective Action
Dark current too low: 0	The dark energy level equals 0.	1. Cycle power to the detector. 2. Contact Waters Technical Service.
Electronic A/D failure	Lamp optimization is adjusted at the minimum level.	Cycle power.
	Data acquisition via A/D converters is interrupt-driven. If interrupt is too long, problem with data acquisition is indicated.	1. Cycle power to the detector. 2. Contact Waters Technical Service.
Filter initialization failure: Erbium position	Unit sensors cannot find erbium filter position.	1. Cycle power to the detector. 2. Contact Waters Technical Service.
Filter initialization failure: No filters found	Unit sensors observe transition to dark before homing the optical filter.	1. Cycle power to the detector. 2. Contact Waters Technical Service.
Filter initialization failure: No reference energy	Unit sensors cannot find any light energy before homing the optical filter.	1. Cycle power to the detector. 2. Contact Waters Technical Service.
Filter initialization failure: No response	Unit sensors cannot identify any dark regions.	1. Cycle power to the detector. 2. Contact Waters Technical Service.
Filter initialization failure: Order filter position	Unit sensors cannot find the order filter position.	1. Cycle power to the detector. 2. Contact Waters Technical Service.

Instrument error messages (Continued)

Error Message	Description	Corrective Action
Filter initialization failure: Shutter position	Unit sensors cannot find the shutter position.	1. Cycle power to the detector. 2. Contact Waters Technical Service.
Grating initialization failure: Backlash too high	Backlash is the difference between the forward and reverse peak positions of deuterium at 656 nm. If this difference is greater than 1 step, the unit displays this message.	1. Cycle power to the detector. 2. Contact Waters Technical Service.
Grating initialization failure: No home sensor	Search for the home sensor failed.	1. Cycle power to the detector. 2. Contact Waters Technical Service.
Lamp data not found	Stored lamp data are invalid.	Cycle power to the detector. This action removes the error.
Method not found	Stored method data are invalid.	Cycle power to the detector. This action removes the error.
Scan not found	Stored scan data are invalid.	Cycle power to the detector. This action removes the error.
System cannot respond	Error occurs while unit is positioning next wavelength or changing modes. Occurs during initialization or calibration.	1. Cycle power to the detector. 2. Contact Waters Technical Service.

Instrument error messages (Continued)

Error Message	Description	Corrective Action
System not calibrated.	The calibration read from nonvolatile memory is not valid.	1. Cycle power to the detector. 2. Perform manual calibration. 3. Contact Waters Technical Service.

User-selected diagnostic tests

Overview

You can run several diagnostic tests for troubleshooting the detector and verifying that its electronics and optics perform correctly.

To perform user-selectable diagnostic tests

1. Press the DIAG key from the detector's front panel.

   #### Diagnostic tests choice list

2. To access a specific diagnostic test, press the up or down arrow key to select a test, and then press Enter.

   Alternative: Press a number on the keypad corresponding to the test number. Choices that display other choices are indicated by >>.

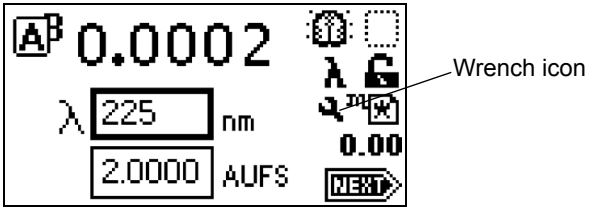
Sticky diagnostic tests remain in effect until you disable them. When a test is active, the detector absorbance screen displays a wrench icon (see the figure below).

You can disable a specific sticky diagnostic test by resetting it to the default settings.

You can disable all active sticky diagnostic tests by pressing DIAG and then selecting “1, Reset instrument.”

If no sticky diagnostic tests are active, the wrench icon does not appear on the absorbance screen. When you power-off the detector, sticky diagnostic tests become inactive.

Absorbance screen with sticky diagnostic tests active



The user-selectable sticky diagnostic tests are:

- Fix (Set) Voltage Output
- Fix (Set) Absorbance Input
- Generate Test Peaks
- Optical Filter Override

Caution: Application of sticky diagnostic tests affects results. To clear changes to the voltage output or absorbance input or to make a manual optical filter change, select 1, “Reset instrument” from the Diagnostics choice list, or cycle power to the detector.

The table below lists diagnostic tests by their number on the choice list (see page 5-11 for more information).

Detector diagnostic tests

Diagnostic test	Description
1. Reset instrument	Resets all diagnostic tests to defaults. Renders sticky diagnostic tests inactive and removes the wrench icon.
2. Sample & ref energy	Makes it possible to view sample and reference energy (displayed in nanoamps) on channel A or B.

Detector diagnostic tests (Continued)

Diagnostic test	Description
3. Input & output >>	List of diagnostic tests to control four contact closure inputs and two switch outputs: • Auto-zero offset • Fix absorbance • Fix voltage • Contact closures & events • Previous choices <<
4. Lamp, display, & keypad >>	List of diagnostic tests used to test lamp, display, and keypad functions: • Change lamp • Test keypad • Test display • Previous choices <<
5. Service	Diagnostic tests used by Waters Service personnel.
6. Other diagnostics >>	Diagnostic tests that generate test peaks, with which you determine wavelength accuracy or override the default filter setting: • Generate test peaks • Optical filter override • Previous choices <<

Using the diagnostic tests

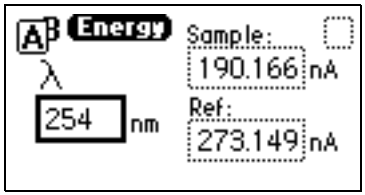
The detector employs both user-selectable and service diagnostic tests. You access user diagnostic tests by pressing the DIAG key. Only qualified Waters service personnel can access service diagnostic tests.

Using the sample and reference energy diagnostic tests

You use the sample and reference energy diagnostic tests to plot the output of the analog channels to examine noise fluctuations and to compare with the

AU time trace. The current sample and reference energy readings appear in nanoamps (nA).

Sample and reference energy diagnostic test



To use the sample and reference energy diagnostic test

1. Press DIAG, and then press 2.
2. Change the wavelength, if necessary.
3. Press Enter.

   Result: When the new wavelength shifts to the left, the corresponding sample and reference energies appear.
4. If you are operating the detector in dual-wavelength mode, press the A/B key to view sample and reference energy on the other wavelength.

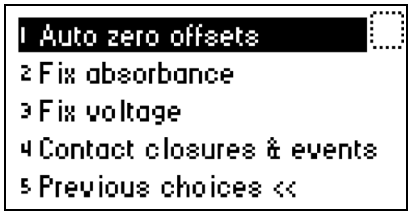
Using the input and output diagnostic tests

You use the input and output diagnostic tests to

- display and reset the autozero offsets.
- fix (set) absorbance.
- fix (set) the voltage on the 2-V output.
- monitor contact closures and toggle event switches.
- generate test peaks.
- override the optical filter.

To perform any of the input and output diagnostic tests, press DIAG, and then press 3, for Input & output. A choice list appears.

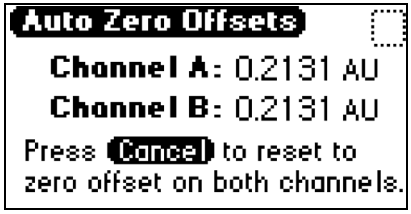
Input & output diagnostic test choice list



Displaying auto-zero offsets

From the Input & Output choice list, press 1 (Auto-zero offsets). This diagnostic test also allows you to display and reset the offset to zero on both channels by pressing Cancel (Shift 0).

Auto-zero offsets diagnostic display

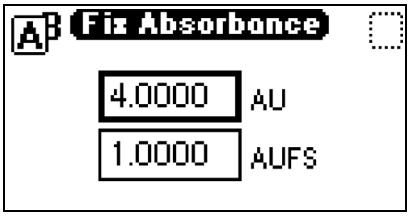


Setting fixed absorbance value

From the Input & Output choice list, press 2 (Fix absorbance) to set a fixed absorbance value for channel A or channel B. The allowable range is from –4.0000 AU to +4.0000 AU. For convenience, you can also specify sensitivity

(AUFS) using this diagnostic test. The allowable AUFS range is from +0.0001 to +4.0000 AUFS.

Fix absorbance diagnostic display

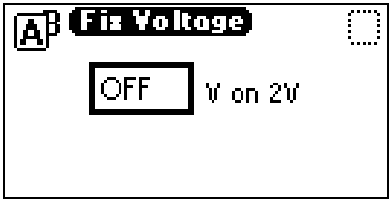


This test sets the voltages on the analog output channels based on the current AUFS setting. This is a sticky diagnostic test.

Setting fixed voltage output

From the Input & Output choice list, press 3 (Fix voltage) to select a voltage for the analog output. You can select a voltage for both output channels in the –0.10 V to +2.10 V range.

Fix Voltage diagnostic display

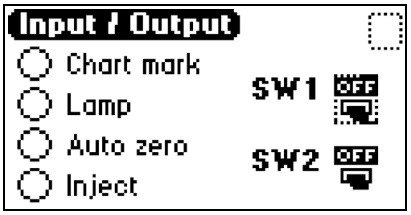


The voltage is driven on the selected analog channel (A or B). This is a sticky diagnostic.

To monitor contact closures and setting switches

1. From the Input & Output choice list, press 4 (Contact closures & events) to monitor the four contact closure inputs and to control the two switch outputs.

   #### Contact closures & events diagnostic display

   The Input & Output diagnostic test allows real-time monitoring of the state of the contact closure inputs. A solid (filled in) circle indicates the contact closure is closed (ON = High). An open (empty) circle indicates the contact closure is open (OFF = Low).

2. For the outputs (SW1 and SW2):

   a. Press Enter to display the active switch (surrounded by a dotted-line border).

   b. Press any numerical key to change the status of the switch (ON to OFF or vice versa).

   c. Press Enter to select the second switch.

Using the lamp, display, and keypad diagnostic tests

To access the lamp, display, and keypad diagnostic tests, press DIAG and then press 4.

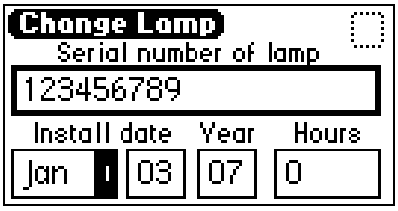
Using the change lamp diagnostic test

Caution: Make sure the detector is powered-off and the power cord is disconnected before you replace the lamp.

To change the lamp

1. From the Lamp, display & keypad choice list, press 1 (Change lamp) to access the Change Lamp diagnostic display.

   #### Change Lamp diagnostic display

2. Enter the 9-digit serial number and date of installation for the new lamp. Press Enter after each entry.

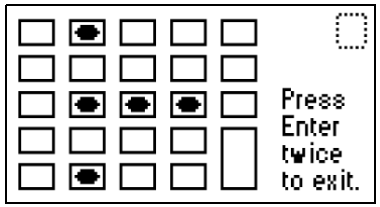
   Tip: Use this function whenever you change the lamp to enter a new serial number and installation date (see page 4-21 and page 4-23 for a complete explanation of the lamp replacement procedure).

   Caution: Neglecting to record the new lamp serial number following the prescribed procedures referenced above voids the lamp warranty. The date of the previous lamp installation remains in the detector memory.

To use the Test keypad diagnostic test

1. From the Lamp, display & keypad choice list, press 2 (Test keypad) to run the keypad test.

   #### Keypad diagnostic test display

2. On the keypad diagnostic test display, press any key to begin the test. Then press each key until you pressed all of them. If the keypad is operating properly, each key location is filled in and then cleared with another press of the key. If any key does not respond when pressed, contact your Waters service representative.

   Rule: You must press Enter twice to exit the keypad diagnostic display.

To run the test display diagnostic test

1. From the Lamp, display & keypad choice list, press 3 (Test display) to run the test.

   Result: The display fills from top to bottom and from right to left, then returns to the Lamp, display & keypad choice list. If the display does not completely fill, either horizontally or vertically, contact your Waters service representative.

2. From the Lamp, display & keypad choice list, press 4.

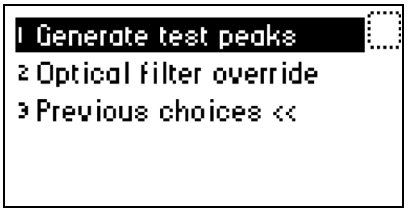
Using the other detector diagnostic tests

The user diagnostic test display provides two additional tests:

- Generate test peaks – Specifies the generation of test peaks to calibrate your chart recorder or data system.
- Manually override the optical filter – Selects a filter different from that specified as part of the detector's normal operating mode.

To run either test, press DIAG and then press 5 (Other diagnostics).

Other diagnostics choice list



To generate test peaks

1. From the Other diagnostics choice list, press 1 (Generate test peaks) to generate test peaks every 100 seconds on the chart, trace, or other output.

   Restriction: The Generate test peaks diagnostic test works only in single-wavelength mode.

   Every 100 seconds, the detector generates an approximate 1-AU peak with a standard deviation of 10 seconds on the trace, chart, or data system display until you disable the Generate test peaks diagnostic test. The amplitude of the test peaks is affected by your choice of filter time-constant.

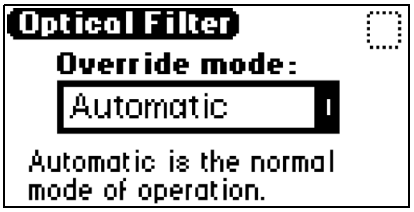
   This is a sticky diagnostic test. When this routine is selected, the choice list changes to read “Disable test peaks.”

2. Press 1 (Disable test peaks) from the Other diagnostics choice list to turn off this diagnostic test.

To override the optical filter

1. From the Other diagnostics choice list, press 2 (Optical filter override) to manually override the detector's automatic filter choice.

Optical Filter Override diagnostic test display



2. In the Optical Filter Override diagnostic test display, press Enter to display the following filter choice list.

Automatic	1
Second Order	2
None	3
Erbium	4
Shutter	5

Tip: The detector normally operates with the filter in the Automatic position. This is a sticky diagnostic test.

3. Press the number corresponding to your filter choice, or leave the default filter choice (Automatic) on.
4. To deactivate this diagnostic test, press DIAG, and then press 1, or select Automatic from the choice list.

Service diagnostic tests

Only qualified Waters service personnel can access the detector service diagnostic tests.

Troubleshooting

This section provides some causes of errors and recommended troubleshooting actions. Keep in mind that the source of apparent detector problems may lie within the chromatography or your other instruments, as well as the detector.

Most detector problems are relatively easy to correct. If you are unable to correct a problem or a failed condition, contact Waters Technical Service.

When you contact Waters

To expedite your request for service, ensure the following information is available when you call Waters Technical Service:

- Detector serial number
- Problem symptom(s)
- Operating wavelength(s)
- AUFS or measurement range
- Flow rate
- Filter setting
- Type of column
- Operating pressure
- Solvent(s)
- System configuration (other components)

Tip: The detector can be configured as part of a system with a 2695 Separations Module, Empower or MassLynx software, or a non-Waters product.

Diagnostic tests

The detector performs some user-selected diagnostic tests to help you troubleshoot basic system problems. (See page 5-11, for diagnostic descriptions and instructions on how to use them.)

Error messages that can appear as you start up or operate the detector and suggested corrective actions are described in the tables on page 5-3 and page 5-6.

Power surges

Power surges, line spikes, and transient energy sources can adversely affect detector operations. Be sure that the electrical supply used for the detector is properly grounded and free from any of these conditions.

Hardware troubleshooting

This section contains general hardware troubleshooting for the detector.

General system troubleshooting

Symptom	Possible cause	Corrective action
Analog output incorrect	AUFS setting changed	Reset the AUFS setting.
Calibration or energy error on startup	Cuvette is installed, or a UV absorber in flow cell	1. Remove the cuvette. 2. Flush the flow cell. 3. Perform manual calibration.
Detector inoperative	Fuse blown	Confirm the front panel display is operational; if not, replace the AC rear panel fuse.
	No power at outlet	Test the outlet by connecting another electrical unit known to be in working order and determine whether it operates.
Deuterium lamp does not light	Faulty lamp	Replace the lamp.
	Lamp not plugged in	Plug in the lamp connector.
	Bad lamp power supply board	Contact Waters Technical Service.
	Lamp switch in the “off” position.	Inspect the rear panel connections or timed event within a method.
Ethernet problems	Bad Ethernet cable.	Replace the Ethernet cable.

General system troubleshooting (Continued)

Symptom	Possible cause	Corrective action
Front panel display fails to illuminate	Broken electrical connection	Inspect electrical connections.
	Fuse blown	Inspect and, if necessary, replace fuse(s).
	Bad LCD or control board	Contact Waters Technical Service.
Front panel displays odd characters	Faulty EPROMs Bad LCD control board	Contact Waters Technical Service.
In dual-wavelength mode, time scale is incorrect	Data rate on controller is > 1 pt/sec	In dual-wavelength mode, you must select a data sampling rate of 1 point per second.
Keypad not functioning	Keypad defective	1. Cycle power to the detector and run the keypad diagnostic test. 2. Contact Waters Technical Service.
No sample and reference energy	Lamp burned out	Attempt to reignite using the lamp key. Replace the lamp.
	Lamp extinguished.	Check the lamp icon. Run the Sample & ref energy diagnostic test.
Peak out of range error at start up	Cuvette in cuvette holder; mobile phase absorbance too high; air bubbles	1. Remove cuvette from cuvette holder. 2. Be sure the flow cell mobile phase does not absorb above 250 nm. 3. Ensure that no air bubbles are in the flow cell. 4. Recalibrate the detector. If problems persist, contact Waters Technical Service.

A Safety Advisories

Waters instruments display hazard symbols designed to alert you to the hidden dangers of operating and maintaining the instruments. Their corresponding user guides also include the hazard symbols, with accompanying text statements describing the hazards and telling you how to avoid them. This appendix presents all the safety symbols and statements that apply to the entire line of Waters products.

Contents

Topic	Page
Warning symbols	A-2
Caution symbol	A-5
Warnings that apply to all Waters instruments	A-5
Electrical and handling symbols	A-12

Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with an instrument's use or misuse. Heed all warnings when you install, repair, and operate Waters instruments. Waters assumes no liability for the failure of those who install, repair, or operate its instruments to comply with any safety precaution.

Task-specific hazard warnings

The following warning symbols alert you to risks that can arise when you operate or maintain an instrument or instrument component. Such risks include burn injuries, electric shocks, ultraviolet radiation exposures, and others.

When the following symbols appear in a manual's narratives or procedures, their accompanying text identifies the specific risk and explains how to avoid it.

Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)

Warning: (Risk of burn injury from contacting hot surfaces.)

Warning: (Risk of electric shock.)

Warning: (Risk of fire.)

Warning: (Risk of needle puncture.)

Warning: (Risk of injury caused by moving machinery.)

Warning: (Risk of exposure to ultraviolet radiation.)

Warning: (Risk of contacting corrosive substances.)

Warning: (Risk of exposure to a toxic substance.)

Warning: (Risk of personal exposure to laser radiation.)

Warning: (Risk of exposure to biological agents that can pose a serious health threat.)

Specific warnings

The following warnings can appear in the user manuals of particular instruments and on labels affixed to them or their component parts.

Burst warning

This warning applies to Waters instruments fitted with nonmetallic tubing.

Warning: Pressurized nonmetallic, or polymer, tubing can burst. Observe these precautions when working around such tubing:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to incompatible compounds like tetrahydrofuran (THF) and nitric or sulfuric acids.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, which significantly reduces the pressure at which the tubing can rupture.

Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.

Warning: Where significant quantities of flammable solvents are involved, a continuous flow of nitrogen into the ion source is required to prevent possible ignition in that enclosed space.

Ensure that the nitrogen supply pressure never falls below 690 kPa (6.9 bar, 100 psi) during an analysis in which flammable solvents are used. Also ensure a gas-fail connection is connected to the LC system so that the LC solvent flow stops if the nitrogen supply fails.

Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.

Warning: To avoid electric shock, do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.

This warning applies to certain instruments when they are in Operate mode.

Warning: High voltages can be present at certain external surfaces of the mass spectrometer when the instrument is in Operate mode. To avoid non-lethal electric shock, make sure the instrument is in Standby mode before touching areas marked with this high voltage warning symbol.

Biohazard warning

This warning applies to Waters instruments that can be used to process material that might contain biohazards: substances that contain biological agents capable of producing harmful effects in humans.

Warning: Waters instruments and software can be used to analyze or process potentially infectious human-sourced products, inactivated microorganisms, and other biological materials. To avoid infection with these agents, assume that all biological fluids are infectious, observe Good Laboratory Practices, and consult your organization's biohazard safety representative regarding their proper use and handling. Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Chemical hazard warning

This warning applies to Waters instruments that can process corrosive, toxic, flammable, or other types of hazardous material.

Warning: Waters instruments can be used to analyze or process potentially hazardous substances. To avoid injury with any of these materials, familiarize yourself with the materials and their hazards, observe Good Laboratory Practices (GLP), and consult your organization's safety representative regarding proper use and handling. Guidelines are provided in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals.*

Caution symbol

The caution symbol signifies that an instrument's use or misuse can damage the instrument or compromise a sample's integrity. The following symbol and its associated statement are typical of the kind that alert you to the risk of damaging the instrument or sample.

Caution: To avoid damage, do not use abrasives or solvents to clean the instrument's case.

Warnings that apply to all Waters instruments

When operating this device, follow standard quality control procedures and the equipment guidelines in this section.

Attention: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Important: Toute modification sur cette unité n'ayant pas été expressément approuvée par l'autorité responsable de la conformité à la réglementation peut annuler le droit de l'utilisateur à exploiter l'équipement.

Achtung: Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.

Avvertenza: qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.

Atencion: cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.

注意：未經有關法規認證部門允許對本設備進行的改變或修改,可能會使使用者喪失操作該設備的權利。

注意：未经有关法规认证部门明确允许对本设备进行的改变或改装，可能会使使用者丧失操作该设备的合法性。

주의: 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.

注意：規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザーとしての承認が無効になる可能性があります。

Warning: Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames.
- Do not use tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.

Attention: Manipulez les tubes en polymère sous pression avec precaution:

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Eteignez toute flamme se trouvant à proximité de l'instrument.
- Evitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Evitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.

Vorsicht: Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:

- In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.
- Alle offenen Flammen in der Nähe löschen.
- Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.
- Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.
- Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.

Attenzione: fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnere tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.

Advertencia: se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Si hubiera alguna llama las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.

警告：當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。

警告：当有压力的情况下使用管线时，小心注意以下几点：

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚枫会导致非金属管线膨胀，大大降低管线的耐压能力。

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸술폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.

警告：圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン(THF)や高濃度の硝酸または硫酸などを流さないでください。
- 塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があり、その場合、チューブは極めて低い圧力で破裂します。

Warning: The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Attention: L'utilisateur doit être informé que si le matériel est utilisé d'une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d'être défectueuses.

Vorsicht: Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwenddung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.

Attenzione: si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.

Advertencia: el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.

警告：使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被消弱。

警告：使用者必须非常清楚如果设备不是按照制造厂商指定的方式使用，那么该设备所提供的保护将被削弱。

경고: 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.

警告：ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。

Warning: To protect against fire, replace fuses with those of the type and rating printed on panels adjacent to instrument fuse covers.

Attention: pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués sur le panneau à proximité du couvercle de la boite à fusible de l'instrument.

Vorsicht: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert auf den Tafeln neben den Sicherungsabdeckungen des Geräts gedruckt sind.

Attenzione: per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate sui pannelli adiacenti alla copertura fusibili dello strumento.

Advertencia: Para evitar incendios, sustituir los fusibles por aquellos del tipo y características impresos en los paneles adyacentes a las cubiertas de los fusibles del instrumento.

警告：為了避免火災，更換保險絲時，請使用與儀器保險絲蓋旁面板上所印刷之相同類型與規格的保險絲。

警告：为了避免火灾，应更换与仪器保险丝盖旁边面板上印刷的类型和规格相同的保险丝。

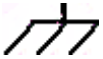
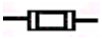
경고: 화재의 위험을 막으려면 기기 퓨즈 커버에 가까운 패널에 인쇄된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

警告：火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

Electrical and handling symbols

Electrical symbols

These can appear in instrument user manuals and on the instrument's front or rear panels.

	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Protective conductor terminal
	Frame, or chassis, terminal
	Fuse
	Recycle symbol: Do not dispose in municipal waste.

Handling symbols

These handling symbols and their associated text can appear on labels affixed to the outer packaging of Waters instrument and component shipments.

	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!

B Specifications

This appendix lists individual operating specifications for the Waters 2489 UV/Visible Detector, as follows:

- Operational specifications
- Optical specifications
- Specifications for the optional flow cells

Contents:

Topic	Page
Operational specifications	B-2
Optical specifications	B-4
Optional Waters TaperSlit flow cell specifications	B-5

Operational specifications

Operational specifications

Condition	Specification
Wavelength range	190 to 700 nm
Bandwidth	<5 nm
Wavelength accuracy	±1.0 nm
Wavelength repeatability	±0.1 nm
Single λ dry noise	<5 µAU (at 230 nm, 1-second digital filter, 30-second segments, 10-Hz data rate, analytical cell)
Wet noise	≤8 µAU (at 230 nm, 1-second digital filter, 30-second segments, 10-Hz data rate, 1-mL/min. flow of acetonitrile, analytical cell)
Dual channel noise (dry)	≤35 µAU (measured at 230 nm, running at 230 to 280 nm with 2-second filter at 1-Hz data rate; dry, analytical cell)
Dual channel noise (wet)	≤45 µAU (measured at 230 nm, running at 230 to 280 nm with 2-second filter at 1-Hz data rate, 1mL/min. flow of acetonitrile, analytical cell)
Linearity	<5% at 2.5 AU, propylparaben at 257 nm
Drift	1.0×10^{-4} AU/hr. max ($\Delta T = \pm 2$ °C/hr.)
Thermal drift	1.0×10^{-4} AU/°C max ($\Delta T = \pm 2$ °C/hr.)
Sensitivity setting range	0.0001 to 4.0000 AUFS
Filter setting range	Single-wavelength: 0.1 to 5.0 seconds Dual-wavelength: 1 to 50 seconds
time-constant	0.0- to 5.0-second Hamming filter
Digital data rates	1, 2, 5, 10, 20, 40, 80 Hz (single channel) 1, 2 Hz (dual channel)

Operational specifications (Continued)

Condition	Specification
Analog output data rates (single λ mode)	10, 20, 40, 80 Hz (channel A) 10 Hz only (channel B)
Optical Component Specifications	
Lamp source	30-W High Brightness Deuterium lamp, 0.5 nm aperture, pre-aligned 2000-hour warranty, front accessible
Photodiodes	2 silicon photodiodes (matched pair)
Second-order filter	Automatic for wavelengths ≥ 370nm
Wavelength calibration filter	Erbium filter, used at startup or on demand
Flow cell	TaperSlit™ flow cell design
Nitrogen purge	Purge fitting present on optics bench
Path length	10 mm (standard analytical)
Cell volume	10 µL (standard analytical)
Pressure limit	6895 kPa (69 bar, 1000 psi)
Materials	316 stainless steel, fused silica, Tygon®
Environmental Specifications	
Operating temperature	4 to 40 °C (39 to 104 °F)
Operating humidity	20% to <95%, noncondensing
Shipping and storage temperature	–30 to +60 °C
Shipping and storage humidity	0% to <95%, noncondensing
Electrical Specifications	
Line frequency	50 to 60 Hz
Line voltage	100 to 240 Vac
Max VA input	185 VA

Operational specifications (Continued)

Condition	Specification
Fuse ratings	Two fuses: 100 to 240-Vac, 50 to 60-Hz F 3.15-A 250-V fast blo, 5 × 20 mm (IEC)
Attenuated analog output channel: 2 VFS	Attenuation range: 0.0001 to 4.000 AU 2-V output range: –0.1 to +2.1 V
Two event outputs	Type: Contact closure Voltage: +30 V Current: 1 A
Four event inputs	Input voltage: +30 V maximum 100-ms minimum period
Dimensions	
Height	20.8 cm (8.2 inches)
Length	50.3 cm (19.8 inches)
Width	28.4 cm (11.2 inches)
Weight	9.3 kg (~20.5 pounds)

Optical specifications

Optical specifications

Condition	Specification
Monochromator type	Fastie-Ebert
Grating	Plane holographic 1800 grooves/mm
Optical bandwidth	5 nm
Lamp power	30 W

Optional Waters TaperSlit flow cell specifications

Optional Waters TaperSlit flow cell specifications

	Volume (µl)	Path length (mm)	Sample tube internal diameter (Inches) In/Out		Pressure rating psi/bar
Analytical cell	10	10	0.009	0.009	1000/70
Semi-prep cell	2.6	3	0.040	0.040	1000/70
Microbore cell	2.6	3	0.005	0.005	1000/70
Inert (titanium) cell	10	10	0.010	0.010	1000/70
High pressure cell for LCMS	10	10	0.009	0.009	3000/210
Variable path length flow cell (VPF)	0.69 to 13.72	0.15 – 3 (factory preset at 0.5 mm)	0.04	0.04	1000/70
Autopurification cell	2.6	1.0	0.009 0.020 0.040	IN 1 IN 2 OUT	2000/140

C Solvent Considerations

Contents:

Topic	Page
Introduction	C-2
Solvent miscibility	C-3
Buffered solvents	C-6
Head height	C-6
Solvent viscosity	C-6
Mobile phase solvent degassing	C-7
Solvent degassing methods	C-8
Wavelength selection	C-9

Warning: To avoid chemical hazards, always observe Good Laboratory Practices when operating your system.

Introduction

Preventing contamination

For information on preventing contamination, refer to *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems* (part number 715001307), or visit www.waters.com.

Clean solvents

Clean solvents provide reproducible results and permit you to operate with minimal instrument maintenance.

A dirty solvent can cause baseline noise and drift. It can also block the solvent filters with particulate matter.

Solvent quality

Use HPLC-grade solvents for the best possible results. Filter solvents through 0.45-µm filters before their use. Solvents distilled in glass generally maintain their purity from lot to lot; use them to ensure the best possible results.

Preparation checklist

The following solvent preparation guidelines help to ensure stable baselines and good resolution:

- Filter solvents with a 0.45-µm filter.
- Degas and/or sparge the solvent.
- Stir the solvent.
- Keep in a place free from drafts and shock.

Water

Use water only from a high-quality water purification system. If the water system does not deliver filtered water, filter it through a 0.45-µm membrane filter before use.

Using buffers

When you use buffers, dissolve salts first, adjust the pH, then filter to remove insoluble material.

Tetrahydrofuran

When using unstabilized tetrahydrofuran, ensure that your solvent is fresh. Previously opened bottles of tetrahydrofuran contain peroxide contaminants, which cause baseline drift.

Warning: Tetrahydrofuran contaminants (peroxides) are potentially explosive if concentrated or taken to dryness.

Solvent miscibility

Before you change solvents, refer to the table below to determine the miscibility of the solvents to be used. When you change solvents, be aware that

- changes involving two miscible solvents may be made directly. Changes involving two solvents that are not totally miscible (for example, from chloroform to water), require an intermediate solvent (such as isopropanol).
- temperature affects solvent miscibility. If you are running a high-temperature application, consider the effect of the higher temperature on solvent solubility.
- buffers dissolved in water can precipitate when mixed with organic solvents.

When you switch from a strong buffer to an organic solvent, flush the buffer out of the system with distilled water before you add the organic solvent.

Solvent miscibility

Polarity index	Solvent	Viscosity CP, 20 °C	Boiling point °C (1 atm)	Miscibility number (M)	λ Cutoff (nm)
–0.3	N-decane	0.92	174.1	29	—
–0.4	Iso-octane	0.50	99.2	29	210
0.0	N-hexane	0.313	68.7	29	—
0.0	Cyclohexane	0.98	80.7	28	210
1.7	Butyl ether	0.70	142.2	26	—
1.8	Triethylamine	0.38	89.5	26	—
2.2	Isopropyl ether	0.33	68.3	—	220
2.3	Toluene	0.59	100.6	23	285
2.4	P-xylene	0.70	138.0	24	290
3.0	Benzene	0.65	80.1	21	280
3.3	Benzyl ether	5.33	288.3	—	—
3.4	Methylene chloride	0.44	39.8	20	245
3.7	Ethylene chloride	0.79	83.5	20	—
3.9	Butyl alcohol	3.00	117.7	—-	—
3.9	Butanol	3.01	177.7	15	—
4.2	Tetrahydrofuran	0.55	66.0	17	220
4.3	Ethyl acetate	0.47	77.1	19	260
4.3	1-propanol	2.30	97.2	15	210
4.3	2-propanol	2.35	117.7	15	—-
4.4	Methyl acetate	0.45	56.3	15, 17	260
4.5	Methyl ethyl ketone	0.43	80.0	17	330
4.5	Cyclohexanone	2.24	155.7	28	210
4.5	Nitrobenzene	2.03	210.8	14, 20	—
4.6	Benzonitrile	1.22	191.1	15, 19	—
4.8	Dioxane	1.54	101.3	17	220

Solvent miscibility (Continued)

Polarity index	Solvent	Viscosity CP, 20 °C	Boiling point °C (1 atm)	Miscibility number (M)	λ Cutoff (nm)
5.2	Ethanol	1.20	78.3	14	210
5.3	Pyridine	0.94	115.3	16	305
5.3	Nitroethane	0.68	114.0	—	—
5.4	Acetone	0.32	56.3	15, 17	330
5.5	Benzyl alcohol	5.80	205.5	13	—
5.7	Methoxyethanol	1.72	124.6	13	—
6.2	Acetonitrile	0.37	81.6	11, 17	190
6.2	Acetic acid	1.26	117.9	14	—
6.4	Dimethylformamide	0.90	153.0	12	—
6.5	Dimethylsulfoxide	2.24	189.0	9	—
6.6	Methanol	0.60	64.7	12	210
7.3	Formamide	3.76	210.5	3	—
9.0	Water	1.00	100.0	—	—

How to use miscibility numbers

Use miscibility numbers (M-numbers) to predict the miscibility of a liquid with a standard solvent (see "Solvent miscibility" on page C-3.)

To predict the miscibility of two liquids, subtract the smaller M-number value from the larger M-number value.

- If the difference between the two M-numbers is 15 or less, the two liquids are miscible in all proportions at 15 °C (59 °F).
- A difference of 16 indicates a critical solution temperature from 25 to 75 °C (77 to 167 °F), with 50 °C (122 °F) as the optimal temperature.
- If the difference is 17 or greater, the liquids are immiscible or their critical solution temperature is above 75 °C (167 °F).

Some solvents prove immiscible with solvents at both ends of the lipophilicity scale. These solvents receive a dual M-number:

- The first number, always lower than 16, indicates the degree of miscibility with highly lipophilic solvents.
- The second number applies to the opposite end of the scale. A large difference between these two numbers indicates a limited range of miscibility.

For example, some fluorocarbons are immiscible with all the standard solvents and have M-numbers of 0, 32. Two liquids with dual M-numbers are usually miscible with each other.

A liquid is classified in the M-number system by testing for miscibility with a sequence of standard solvents. A correction term of 15 units is then either added or subtracted from the cutoff point for miscibility.

Buffered solvents

When using a buffer, use a good quality reagent and filter it through a 0.45-µm filter.

Do not leave the buffer stored in the system after use. Flush all fluid paths with HPLC-quality water before shutting the system down and leave distilled water in the system (flush with 90% HPLC-quality water: 10% methanol for shutdowns scheduled to be more than one day). Use a minimum of 15 mL for sparge-equipped units, and a minimum of 45 mL for in-line vacuum degasser-equipped units.

Head height

Position the solvent reservoirs at a level above the HPLC equipment or on top of the pump or detector (with adequate spill protection).

Solvent viscosity

Generally, viscosity is not important when you are operating with a single solvent or under low pressure. However, when you are running a gradient, the viscosity changes that occur as the solvents are mixed in different proportions can result in pressure changes during the run. For example, a 1:1 mixture of

water and methanol produces twice the pressure of either water or methanol alone.

If the extent to which the pressure changes will affect the analysis is not known, monitor the pressure during the run using the Chart Out terminal.

Mobile phase solvent degassing

Mobile phase difficulties account for 70% or more of all liquid chromatographic problems. Using degassed solvents is important, especially at wavelengths below 220 nm.

Degassing provides

- stable baselines and enhanced sensitivity.
- reproducible retention times for eluting peaks.
- reproducible injection volumes for quantitation.
- stable pump operation.

Gas solubility

Only a finite amount of gas can be dissolved in a given volume of liquid. This amount depends on

- the chemical affinity of the gas for the liquid.
- the temperature of the liquid.
- the pressure applied to the liquid.

Changes in the composition, temperature, or pressure of the mobile phase can all lead to outgassing.

Effects of intermolecular forces

Nonpolar gases (N_2, O_2, CO_2, He) are more soluble in nonpolar solvents than in polar solvents. Generally, a gas is most soluble in a solvent with intermolecular attractive forces similar to those in the gas (like dissolves like).

Effects of temperature

Temperature affects the solubility of gases. If the heat of solution is exothermic, the solubility of the gas decreases when you heat the solvent. If

the heat of solution is endothermic, the solubility increases when you heat the solvent. For example, the solubility of He in H_2O decreases with an increase in temperature, but the solubility of He in benzene increases with an increase in temperature.

Effects of partial pressure

The mass of gas dissolved in a given volume of solvent is proportional to the partial pressure of the gas in the vapor phase of the solvent. If you decrease the partial pressure of the gas, the amount of that gas in solution also decreases.

Solvent degassing methods

This section describes the solvent degassing techniques that will help you to attain a stable baseline. Degassing your solvent also improves reproducibility and pump performance.

You can use either of the following methods to degas solvents:

- Sparging with helium
- Vacuum degassing

Sparging

Sparging removes gases from solution by displacing dissolved gases in the solvent with a less soluble gas, usually helium. Well-sparged solvent improves pump performance. Helium sparging brings the solvent to a state of equilibrium, which may be maintained by slow sparging or by keeping a blanket of helium over the solvent. Blanketing inhibits reabsorption of atmospheric gases.

Sparging can change the composition of mixed solvents.

Vacuum degassing

The in-line vacuum degasser operates on the principle of Henry's Law to remove dissolved gases from the solvent. Henry's Law states that the mole fraction of a gas dissolved in liquid is proportional to the partial pressure of that gas in the vapor phase above the liquid. If the partial pressure of a gas on the surface of the liquid is reduced, for example, by evacuation, then a proportional amount of that gas comes out of solution.

Vacuum degassing can change the composition of mixed solvents.

Solvent degassing considerations

Select the most efficient degassing operation for your application. To remove dissolved gas quickly, consider the following.

Sparging

Helium sparging gives stable baselines and better sensitivity than sonication in a detector, and prevents reabsorption of atmospheric gases. Use this method to retard oxidation when you are using tetrahydrofuran or other peroxide-forming solvents.

Vacuum degassing

The longer the solvent is exposed to the vacuum, the more dissolved gases are removed. Two factors affect the amount of time the solvent is exposed to the vacuum:

- Flow rate – At low flow rates, most of the dissolved gas is removed as the solvent passes through the vacuum chamber. At higher flow rates, lesser amounts of gas per unit volume of solvent are removed.
- Surface area of the degassing membrane – The length of the degassing membrane is fixed in each vacuum chamber. To increase the length of membrane, you can connect two or more vacuum chambers in series.

The in-line degasser is available as an option or factory-installed in the Waters 2695 Separations Module, XE model.

Wavelength selection

This section includes UV cutoff ranges for

- common solvents.
- common mixed mobile phases.
- chromophores.

UV cutoffs for common solvents

The table below shows the UV cutoff (the wavelength at which the absorbance of the solvent is equal to 1 AU) for some common chromatographic solvents. Operating at a wavelength near or below the cutoff increases baseline noise because of the absorbance of the solvent.

UV cutoff wavelengths for common chromatographic solvents

Solvent	UV cutoff (nm)	Solvent	UV cutoff (nm)
1-Nitropropane	380	Ethylene glycol	210
2-Butoxyethanol	220	Iso-octane	215
Acetone	330	Isopropanol	205
Acetonitrile	190	Isopropyl chloride	225
Amyl alcohol	210	Isopropyl ether	220
Amyl chloride	225	Methanol	205
Benzene	280	Methyl acetate	260
Carbon disulfide	380	Methyl ethyl ketone	330
Carbon tetrachloride	265	Methyl isobutyl ketone	334
Chloroform	245	Methylene chloride	233
Cyclohexane	200	*n*-Pentane	190
Cyclopentane	200	*n*-Propanol	210
Diethyl amine	275	*n*-Propyl chloride	225
Dioxane	215	Nitromethane	380
Ethanol	210	Petroleum ether	210
Ethyl acetate	256	Pyridine	330
Ethyl ether	220	Tetrahydrofuran	230
Ethyl sulfide	290	Toluene	285
Ethylene dichloride	230	Xylene	290

Mixed mobile phases

The table below contains approximate wavelength cutoffs for some other solvents, buffers, detergents, and mobile phases. The solvent concentrations

represented are those most commonly used. If you want to use a different concentration, you can determine approximate absorbance using Beer's Law, because absorbance is proportional to concentration.

Wavelength cutoffs for different mobile phases

Mobile phase	UV cutoff (nm)	Mobile phase	UV cutoff (nm)
Acetic acid, 1%	230	Sodium chloride, 1 M	207
Ammonium acetate, 10 mM	205	Sodium citrate, 10 mM	225
Ammonium bicarbonate, 10 mM	190	Sodium dodecyl sulfate	190
BRIJ 35, 0.1%	190	Sodium formate, 10 mM	200
CHAPS, 0.1%	215	Triethyl amine, 1%	235
Diammonium phosphate, 50 mM	205	Trifluoracetic acid, 0.1%	190
EDTA, disodium, 1 mM	190	TRIS HCl, 20 mM, pH 7.0, pH 8.0	202, 212
HEPES, 10 mM, pH 7.6	225	Triton-X™ 100, 0.1%	240
Hydrochloric acid, 0.1%	190	Waters PIC® Reagent A, 1 vial/liter	200
MES, 10 mM, pH 6.0	215	Waters PIC Reagent B-6, 1 vial/liter	225
Potassium phosphate, monobasic, 10 mM dibasic, 10 mM	190 190	Waters PIC Reagent B-6, low UV, 1 vial/liter	190
Sodium acetate, 10 mM	205	Waters PIC Reagent D-4, 1 vial/liter	190

Wavelength selection for chromophore detection

Certain functional groups found in most compounds absorb light selectively. These groups, known as chromophores, and their behavior can be used to categorize the detection of sample molecules.

The table below lists some common chromophores, and their detection wavelengths (λ_{max}), as well as the molar absorptivity (ε_{max}) of each group. Use this information as a guide to select the optimal operating wavelength for a particular analysis. Because of the diversity possible within a given sample, scanning over a range of wavelengths may be necessary to determine the best wavelength for a particular analysis.

Electronic absorption bands of representative chromophores*

Chromophore	Chemical configuration	λ_{max} (nm)	$\in_{max}$ (L/m/cm)	λ_{max} (nm)	$\in_{max}$ (L/m/cm)
Ether	—O—	185	1000		
Thioether	—S—	194	4600	215	1600
Amine	—NH_2	195	2800		
Thiol	—SH	195	1400		
Disulfide	—S—S—	194	5500	255	400
Bromide	—Br	208	300		
Iodide	—I	260	400		
Nitrile	—C≡N	160	—		
Acetylide	—C≡C—	175-180	6000		
Sulfone	—SO_2 —	180	—		
Oxime	—NOH	190	5000		
Azido	>C=N—	190	5000		
Ethylene	—C=C—	190	8000		
Ketone	>C=O	195	1000	270-285	18-30
Thioketone	>C=S	205	strong		
Esters	—COOR	205	50		
Aldehyde	—CHO	210	strong	280-300	11-18
Carboxyl	—COOH	200-210	50-70		
Sulfoxide	>S→O	210	1500		
Nitro	—NO_2	210	strong		
Nitrile	—ONO	220-230	1000-2000	300-400	10
Azo	—N=N—	285-400	3-25		

Electronic absorption bands of representative chromophores* (Continued)

Chromophore	Chemical configuration	λ_{max} (nm)	$\in_{max}$ (L/m/cm)	λ_{max} (nm)	$\in_{max}$ (L/m/cm)
Nitroso	—N=O	302	100		
Nitrate	—ONO_2	270 (shoulder)	12		
Allene	—$(C=C)_2$— (acyclic)	210-230	21,000		
Allene	—$(C=C)_3$—	260	35,000		
Allene	—$(C=C)_4$—	300	52,000		
Allene	—$(C=C)_5$—	330	118,000		
Allene	—$(C=C)_2$— (alicyclic)	230-260	3000-8000		
Ethylenic/ Acetylenic	C=C—C≡C	219	6,500		
Ethylenic/ Amido	C=C—C=N	220	23,000		
Ethylenic/ Carbonyl	C=C—C=O	210-250	10,000-20,000		
Ethylenic/ Nitro	C=C—NO_2	229	9,500		

*Willard, H. H. and others. *Instrumental Methods of Analysis*, 6th ed. Litton Educational Publishing, Inc., 1981. Reprinted by permission of Wadsworth Publishing Co., Belmont, California, 94002.

Index

Symbols

+/- key 3-12
? key 3-9, 3-25
• key 3-12

Numerics

2695 separations module
  connecting to 2-17–2-19
  generating a chart mark from 2-18
  generating an auto-zero on inject from 2-17
  starting a method from 2-15
  turning the lamp on or off from 2-16
600 Series Pump. See Waters 600 Series Pump

A

A/B key 3-4, 3-10
absorbance
  difference plot 3-16
  function 3-14
  halted by fatal error 5-5
  icon 3-5
  MaxPlot function 3-15
  offset parameter 3-13
  RatioPlot function 3-15
  threshold events 3-36–3-39
  threshold timed event parameter 3-36
  trace 3-10
absorbance screen
  display 3-3, 3-4, 3-9
  error message 5-5
  icons 3-5
  navigating from 3-13
  secondary functions 3-14
accessing secondary functions 3-13
activating a pulse or rectangular wave 3-24
active method 3-40
additional functions in dual wavelength mode 3-31
additional peaks 3-32
adjusting
  analog signal 3-16
  contrast 3-24
advancing to the next field 3-12
algorithms 1-9
analog outputs
  channel outputs 2-9
  connections 2-20
  dual wavelength 3-15
  dual wavelength signal 3-19
  signal adjusting 3-14
  single wavelength 3-14
  specifications 3-13
analytes
  additional information on 1-11
  comparing 1-14
  dissolved 1-13
  in cuvette operations 1-13
Anthracene 3-43, 3-53
audience and purpose v
AUFS
  parameter 3-18
  sensitivity 3-44
auto zero
  connections to 600 series pump 2-29
  function 3-17
  on inject parameter 3-17
Auto Zero key 3-9

automatic second-order filter 1-3, 1-4, 1-10
auto-zero
configuring 3-23
connections to 717plus autosampler 2-32
function 3-9, 3-17
offset diagnostic test 5-11, 5-13
on inject parameter 3-17, 3-19
on wavelength changes 3-17
on wavelength changes parameter 3-19
timed event parameter 3-35

B

bandwidth specification B-2
beamsplitter 1-4
benefits of degassing C-8
biohazard warning A-4
buffered mobile phase
flushing 4-5
removing 3-62
buffered solvents C-6
burst warning A-3

C

Calibrate key 3-11, 3-30, 4-17
calibration
algorithm 1-9
errors during startup 3-30
manual 3-11, 3-30
Cancel key 3-11
caution symbol A-5
CE key 3-12
Change Lamp diagnostic test 5-11, 5-16
changing
channels 3-10
contrast 3-11
filter time-constant 3-13
modes 3-32
scale on an absorbance trace 3-10
time- constant 3-13
wavelength, from dual to single 3-9, 3-32
wavelength, from single to dual 3-9, 3-32
channel
changing 3-10
I and II outputs 2-9
on 3-5
selector 3-5
chart mark
configuring event inputs 3-23
generating 2-27, 3-9
generating from the 2695 separations module 2-18
timed event parameter 3-35
Waters 600 Series Pump connections 2-30
Chart Mark key 3-9
chart polarity
function 3-13
parameter 3-19
chart recorder connections 2-25–2-27
charting
difference plot 3-16
MaxPlot function 3-15
RatioPlot function 3-15
checking peak response 3-29
chemical hazard warning A-5
cleaning the flow cell 4-5–4-16
Clear Field key 3-12
clearing
editing changes 3-12
events 3-41
configuration screens 3-22
CONFIGURE key 3-10, 3-22, 3-23
configuring

auto-zero event input 3-23
detector 3-10, 3-22
event inputs 3-23
lamp signal 2-28
with other systems 1-2
connecting to
2695 separations module 2-17–2-19
chart recorder 2-25–2-27
electrical power 2-9–2-10
Empower 2-20–2-23
eSAT/IN module 2-20–2-23
external devices 2-10
fraction collector 2-33
HPLC system 2-6–2-8
other equipment 2-19–2-34
other systems using Ethernet 2-13–2-14
Waters 600 series pump 2-28–2-31
Waters 717plus Autosampler 2-31–2-33
Waters 745/745B/746 Data Module 2-23–2-25
conserving lamp life 3-60
contact closures
and events diagnostic test 5-11
configuring event inputs 3-23
monitoring 5-15
contacting Waters Technical Service 2-5, 4-2, 5-20
contamination
flow cell 3-28
solvent 3-28
contamination, preventing C-2
context-sensitive help 3-25
contrast
adjusting 3-24
changing 3-11
function 3-24
Contrast key 3-11
controlling
from Waters data systems 2-13
cover, removing 4-4
current method conditions 3-13, 3-34, 3-41
cuvette
description 3-56
removing 3-59
replacing holder 3-59
cuvette holder
illustrated 3-57
removing 3-58
replacing 3-59
cuvette option
description 1-13
qualification features 1-3
sample analysis 1-3
scanning 3-42, 3-56–3-59
using 1-4

D

damage
to the detector 2-5
to the flow cell 4-15
damage, reporting 4-2
data system control 2-13
decimal point key 3-12
default Ethernet address 3-22
degassing
benefits C-8
considerations C-9
solvents 3-26, 4-5
deleting a timed event 3-37
design
electronic 1-4
optical 1-4
detector
as spectrophotometer 1-3
damage 2-5

description 1-2–1-3
diagnostic tests 5-9–5-19
mounting 2-4
operating 3-26–3-62
operating under remote control 3-35
optics 1-5
powering-off 3-62
setting up to run 3-13
site requirements for 2-3
specifications B-1–B-5
startup procedures 3-2
troubleshooting 5-20
unpacking and inspecting 2-5
warming up 3-28
deuterium lamp
installing 4-22
optics 1-4
replacement 4-16
DIAG key 3-10
diagnostic tests
auto-zero offset 5-11, 5-13
change lamp 5-11, 5-16
contact closures and events 5-11
DIAG key 3-10
display test 5-11
failure 3-3, 5-2
fix (set) absorbance 5-11
fix (set) voltage 5-11
generating test peaks 5-11, 5-17
input and output 5-11, 5-12
keypad test 5-11, 5-17
lamp, display, and keypad 5-11, 5-15
optical filter override 5-11
procedure 5-9–5-19
reset 5-10
sample and reference energy 5-10, 5-11
service 5-11, 5-19
setting a fixed absorbance value 5-13
setting a fixed voltage output 5-14
startup 3-2
sticky 3-6, 5-9
test display 5-17
user-selected 5-9–5-19
using 5-1–5-22
verification failure 3-28
difference plot 3-16
diffraction grating 1-4
dimensions B-4
dirty flow cell 4-5
disabling
external events 3-13
inputs 3-13
disassembling the flow cell 4-8–4-16
display
absorbance 3-4
absorbance trace 3-10
diagnostic test 5-11, 5-15
lamp use statistics 3-11
options 3-10
system information 3-25
test 5-17
display diagnostic test 5-17
drift specification B-2
dual wavelength mode
changing to single 3-32
description 1-11, 3-9
functions 3-31
key 3-9
operating in 3-31–3-34
parameters 1-12, 3-15

E

EC Authorized Representative vii
electrical

connections 2-9–2-10
specifications B-3
electrical symbols A-12
Empower, controlling the detector using 2-14
enabling
chart mark event inputs 3-23
external events 3-13
inputs 3-13
ending wavelength 3-42
Enter key 3-12
entering negative numbers 3-12
entrance slit 1-4
environmental specifications 2-3, B-3
equipment guidelines v, A-5
Erbium scan 3-44
error messages 5-1–5-22
errors
calibration 3-30
fatal 5-5
startup 3-30, 5-2
eSAT/IN module, connecting to 2-20–2-23
Ethernet
connections 2-13–2-14
default address 3-22
interface 2-13–2-14
event inputs
auto-zero 3-23
chart mark 3-23
configuring 3-23
default 3-23
functions 2-10
inject start 3-23
lamp 3-24
external events
disabling 3-13
enabling 3-13

F

failure of startup diagnostic tests 3-3
features
MaxPlot function 1-14
RatioPlot function 1-14
spectral 1-9
filter time- constant
changing 3-13
filter time-constant
function 3-16
parameter 3-19
filters
filter setting specification B-2
noise 1-7
optical override 5-11
replacing solvent reservoir 4-5
second-order 1-3, 1-4, 1-10
time-constant 3-16
fix (set) absorbance diagnostic test 5-11
fix (set) voltage diagnostic test 5-11
flammable solvents A-3
flow cell
cleaning 4-5–4-16
conditions 3-57
contamination 3-28
damaged 4-13, 4-15
description 1-6–1-8
dirty 4-5
disassembling 4-8–4-16
exploded view 4-13
flushing 4-6
frontal view 4-11
gasket 4-13
head-on view 4-11
inspecting 4-5–4-16
lens 4-13
optics 1-4
optional 4-15
reassembling 4-8–4-16

rebuild kit 4-11
rebuilding 4-14–4-15
removing 4-7
replacing 4-15–4-16
replacing parts 4-5–4-16
scanning 3-42, 3-59
specifications 4-15, B-5
static 3-59
tools required to remove, clean, or replace 4-8
window 4-13
flow cell assembly, removing 4-9
flow cell rebuild kit 4-11
flushing
buffered mobile phase 4-5
flow cell 4-6
fraction collector, connecting to 2-33
functions
absorbance 3-14
analog outputs, dual wavelength 3-15
analog outputs, single wavelength 3-14
auto zero 3-17
auto-zero 3-17
auto-zero on wavelength changes 3-17
dual wavelength mode 3-31
filter time-constant 3-16
MaxPlot 3-15, 3-34
primary 3-14
RatioPlot 3-15, 3-33
scale 3-20–3-22
secondary 3-14
time-constant 3-16
trace 3-20–3-22
zoom 3-20
fuse
holder 4-25
replacing 4-25–4-26

G

gas solubility C-7–C-8
generate test peak diagnostic test 5-11
generating
auto-zero on inject from the 2695 2-17
chart mark from the 2695 2-18
chart marks 2-27, 3-9
spectra 3-9
test peaks 5-17
tick marks 3-44
grating, diffraction 1-4

H

handling symbols A-13
help key 3-9, 3-25
HOME key 3-4, 3-9, 3-13
HOME screen. See absorbance screen

I

I/O signals 2-12
icons
absorbance 3-5
channel on 3-5
channel selector 3-5
keypad lock 3-6
keypad unlock 3-6
lamp off 3-5
lamp on 3-5
method number 3-34
next 3-6
run time 3-6
Sensitivity 3-5
shift 3-6
sticky diagnostic tests 3-6
table of 3-5
wavelength 3-5
wrench 3-6

idle mode 3-3
inaccurate peak areas 3-32
initial method conditions 3-9, 3-23, 3-40
initializing the detector 3-2
initiating a scan 3-9
inject signal 3-23
Inject Start
  Waters 600 Series Pump connections 2-31
  Waters 717plus Autosampler connections 2-32
input and output diagnostic tests 5-11, 5-12
inputs
  disabling 3-13
  enabling 3-13
  signals 2-12
inspecting
  detector 2-5
  flow cells 4-5
installation site selection 2-3
installing a new lamp 4-21–4-23
intended use v
interface bus 2-13–2-14
inverting the chart 3-13
ISM classification vi

K

keypad
  +/- key 3-12
  ? key 3-9, 3-25
  • key 3-12
  A/B key 3-4, 3-10
  Auto Zero key 3-9
  Calibrate key 3-11, 3-30, 4-17
  Cancel key 3-11
  CE key 3-12
  Chart Mark key 3-9
  Clear Field key 3-12
  CONFIGURE key 3-10, 3-23
  Contrast key 3-11
  decimal point key 3-12
  description 3-9
  DIAG key 3-10
  diagnostic test 5-11, 5-17
  Enter key 3-12
  functions 3-7, 3-9
  help key 3-9, 3-25
  HOME key 3-9
  λ/λλ key 3-9, 3-31, 3-32
  Lamp key 3-11
  lamp, display, and keypad diagnostic tests 5-11, 5-15
  Lock key 3-11
  locking 3-11
  METHOD key 3-10, 3-36
  Next key 3-10
  numerical keys 3-10
  Previous key 3-10
  Reset key 3-9
  Run/Stop key 3-9
  Scale key 3-10, 3-20
  SCAN key 3-9, 3-45
  Shift key 3-10
  System Info key 3-11
  TRACE key 3-10, 3-20
  up/down arrow keys 3-9
  using 3-7–3-12
keypad lock icon 3-6
keypad unlock icon 3-6

L

λ/ key 3-31
λ/λλ key 3-9, 3-32
laboratory acquisition and control environment (LAC/E) 2-20
lamp
  change 5-16

Change Lamp diagnostic test 5-11, 5-16
configuring lamp event inputs 3-24
conserving lamp life 3-60
energy and performance 4-17
installing 4-21–4-23
lamp, display, and keypad diagnostic tests 5-11, 5-15
monitoring aging 3-28
new 4-21–4-23
removing 4-18–4-21
replacing 3-28, 4-16–4-23
serial number 4-23–4-24
timed event parameter 3-35
turning off 3-60–3-62
turning on or off 3-11
turning on or off from the separations module 2-16
turning on or off manually 3-60
use statistics 3-11
warranty 4-23
when to replace 4-18
Lamp key 3-11
lamp off icon 3-5
lamp on icon 3-5
line spikes 5-21
linearity specification B-2
locating the detector 2-3
lock icon 3-6
Lock key 3-11
locking the keypad 3-11
loss of current method conditions 3-41

M

maintenance
routine 4-5
maintenance considerations 4-2
manual calibration 3-11, 3-30
manual lamp procedures 3-60
mass spectrometer shock hazard A-4
maximum ratio
field 3-33
function 3-16
parameter 3-19
MaxPlot function
charting 3-15
features 1-14
obtaining 3-34
method
active 3-40
choice list 3-10
current conditions 3-13
initial conditions 3-23, 3-40
Method * 3-34, 3-40
preventing loss of current conditions 3-41
programming 3-34–3-42
resetting a stored 3-41
retrieving 3-40
storing 3-34, 3-39
viewing events within 3-40
METHOD key 3-10, 3-36
method number icon 3-34
minimum AU 3-15, 3-33
minimum ratio
field 3-33
function 3-15
parameter 3-19
miscibility of solvents C-3–C-6
mobile phase
flushing 4-5
removing 3-62
modes, changing 3-32
monitoring
contact closures 5-15
lamp aging 3-28
mounting the detector 2-4
moving to the last entry in a list 3-12

N

navigating in reverse order 3-10
negative number entry 3-12
new timed event 3-36
Next arrow 3-10
Next icon 3-6
Next key 3-10
noise
  filtering 1-7, 3-16
numerical keys 3-10

O

obtaining
  MaxPlot 3-34
  RatioPlot 3-33
  stored spectrum information 3-54
offset
  absorbance 3-13
  Auto-Zero Offset diagnostic test 5-11
  voltage 3-13
operating
  as a stand-alone instrument 3-26
  detector 3-26–3-62
  in dual wavelength mode 1-11, 3-31–3-34
  in single wavelength mode 1-10–1-11, 3-30–3-31
  modes 3-26
  specifications B-1–B-5
  trace and scale functions 3-20–3-22
  under remote control 3-27, 3-35
operator interface 3-4
optical
  and electronic design 1-4
  component specifications B-3
  detector specifications B-4
  filter override diagnostic test 5-11
optics 1-4
optional flow cells 4-15
other equipment
  configuring with 1-2
  connecting to 2-19–2-34
  Ethernet connections 2-13–2-14
output
  connections 2-9
  signals 2-12

P

pace 3-42
parameters
  absorbance threshold timed event 3-36
  analog out (dual wavelength) 3-19
  AUFS 3-18
  auto zero on inject 3-17
  auto-zero on inject 3-17, 3-19
  auto-zero on wavelength changes 3-19
  auto-zero timed event 3-35
  chart mark timed event 3-35
  chart polarity 3-19
  dual wavelength mode 1-12
  filter time-constant 3-19
  lamp timed event 3-35
  maximum ratio 3-19
  minimum ratio 3-19
  polarity timed event 3-35
  primary 3-18
  ratio minimum AU 3-19
  sample and zero scan 3-47
  sample scan 3-45–3-49
  secondary 3-18
  sensitivity timed event 3-35
  single wavelength mode 1-10–1-11
  SW1 timed event 3-35
  SW2 timed event 3-36
  time-constant timed event 3-35

timed event 3-35
voltage offset 3-19
wavelength 3-18
wavelength timed event 3-35
zero scan 3-45
peak response test 3-29
peaks, generating test 5-11, 5-17
performing verification procedures 3-27–3-29
photodiodes 1-4
plumbing connections 2-6–2-8
Plus/minus key 3-12
polarity timed event parameter 3-35
polarity, chart 3-13
power
requirements 2-4
surges 5-21
powering-off the detector 3-62
preventing loss of current method conditions 3-41
Previous key 3-10
primary functions 3-14, 3-18
principles of operation 1-1–1-14
programming
absorbance threshold events 3-37–3-39
switches 3-24
threshold events 3-37–3-39
timed events and methods 3-34–3-42
pulse periods, setting 3-24
purpose and audience v

Q

qualification using the cuvette 1-3

R

ratio minimum AU parameter 3-19
RatioPlot function
charting 3-15
comparing analytes using 1-14
maximum ratio 3-16
minimum AU 3-15
minimum ratio 3-15
obtaining 3-33
rear panel
fuses 4-25
illustration 2-9
signal connections 2-10
reassembling the flow cell 4-8–4-16
rebuild kit, flow cell 4-11
rebuilding the flow cell 4-14–4-15
recalling the absorbance screen using the HOME key 3-4
recording
new lamp serial number 4-23–4-24
sample and reference energy 3-28
rectangular wave signal 3-24
reduction in resolution 3-43
reference
energy 3-45
photodiode 1-4
remote control 3-27, 3-35
removing
buffered mobile phase 3-62
cuvette 3-59
cuvette holder 3-58
flow cell 4-7
flow cell assembly 4-9
front left panel cover 4-4
lamp 4-18–4-21
repeatability specifications B-2
replacing
cuvette holder 3-59
flow cell 4-15–4-16
flow cell parts 4-5, 4-13
front left panel cover 4-4
fuses 4-25–4-26
lamp 4-16–4-23

solvent reservoir filters 4-5
replaying a spectrum 3-56
reservoirs, positioning C-6
reset diagnostic tests 5-10
reset instrument 5-10
Reset key 3-9
resetting
run clock 3-9
stored method 3-41
resolution, reduction in 3-43
retrieving a method 3-40
returning to initial conditions 3-9
reviewing a scan 3-55
routine maintenance 4-5
run clock, stopping 3-9
run time icon 3-6
Run/Stop key 3-9
running
new scan 3-46–3-53
sample scan 3-50
solvent degasser 3-26
zero scan 3-47

S

safety advisories A-1
safety considerations, maintenance 4-3
sample and reference energy diagnostic test 5-10, 5-11
sample energy 3-28, 3-45
sample photodiode 1-4
sample scan
definition 1-13
parameters 3-47
procedure 3-45–3-49
running 3-50
screens 3-49
when to run 3-45
scale
function 3-20–3-22
zooming 3-51
Scale key 3-10, 3-20
scaling factor 3-10
SCAN key 3-9, 3-45
Scanning
subtracting 3-55
scanning
Anthracene 3-43, 3-53
AUFS 3-44
cuvette 3-42, 3-56–3-59
Erbium 3-44
flow cell 3-42, 3-59
initiating 3-9
new spectra 3-46–3-53
pace 3-42
reference energy 3-45
replaying a spectrum 3-56
resolution 3-43
reviewing a scan 3-55
running a sample scan 3-50
sample energy 3-45
sample scan 3-45–3-49
screens 3-49
sensitivity 3-44
spectra 3-42–3-60
storing a scan 3-53
tick marks 3-44
timing 3-45
using the cuvette 3-56–3-59
zero scan 3-45, 3-47
screens
absorbance 3-3, 3-4
HOME 3-3
secondary function screen 3-14
secondary functions 3-13, 3-14, 3-18
second-order filter 1-3, 1-4, 1-10
selecting a location for the detector 2-3
Sensitivity
icon 3-5
sensitivity

AUFS parameter 3-18
scanning 3-44
setting specification B-2
timed event parameter 3-35
serial number
instrument 2-6
lamp 4-23–4-24
service
diagnostic tests 5-11, 5-19
Set absorbance diagnostic test 5-11
set voltage diagnostic test 5-11
setting
detector up to run 3-13
fixed absorbance value 5-13
fixed voltage output 5-14
pulse periods 3-24
switches 5-15
shift icon 3-6
Shift key 3-10
shutting down the detector 3-62
signal
connections 2-10–2-13
input 2-12
output 2-12
start of run 3-23
single pulse signal 3-24
single wavelength mode
changing to dual 3-32
description 3-9
key 3-9
operating in 1-10–1-11, 3-30–3-31
parameters 3-14
site requirements 2-3
slit, entrance 1-4
solvent
buffered solvents C-6
contamination 3-28
degasser 3-26
degassing 4-5
filter 4-5
general considerations C-2–C-3
guidelines C-2
miscibility C-3–C-6
reservoirs C-6
UV cutoff C-9–C-11
viscosity considerations C-6
spare parts 4-3
sparging C-8
specifications
bandwidth B-2
dimensions B-4
drift B-2
electrical B-3
environmental B-3
filter setting B-2
flow cell 4-15, B-5
linearity B-2
operating B-1–B-5
optical B-4
optical component B-3
repeatability B-2
sensitivity setting B-2
wavelength accuracy B-2
wavelength range B-2
spectra
generating 3-9
new 3-46–3-53
obtaining information about 3-54
replaying 3-56
reviewing 3-55
scanning 3-42–3-60
scanning using a static flow cell 3-59
storing 3-53
subtracting 3-55
spectral features 1-9
spectrum scanning 1-12
stand-alone operation 3-26

starting
detector 3-2
methods from the separations module 2-15
run 3-23
run clock 3-9
wavelength 3-42
startup
diagnostic tests 3-2
diagnostic tests failure 3-3
errors 3-30, 5-2
kit 2-24, 2-26
sticky diagnostic tests 3-6, 5-9
stopping the run clock 3-9
stored spectra
obtaining information 3-54
reviewing information 3-55
storing
methods 3-34, 3-39
spectra 3-53
subtracting a spectrum 3-55
switch 1 timed event parameter 3-35
switch 2 timed event parameter 3-36
switched outputs 2-10
switches
programming 3-24
setting 5-15
symbols
caution A-5
electrical A-12
handling A-13
warning A-2
system
displaying information 3-11
information 3-25
specifications B-1–B-5
System Info key 3-11

T

Technical service 5-20
test peaks, generating 5-17
The 4-15
theory of operation 1-1–1-14
threshold events
clearing 3-41
programming 3-37–3-39
tick marks, generating 3-44
time-constant
changing 3-13
function 3-16
timed event parameter 3-35
timed events
and methods 3-34–3-42
clearing 3-41
deleting 3-37
description 3-35
parameters 3-35, 3-36
programming 3-34–3-42
programming a new event 3-36
toggling between channels 3-4
tools to remove, clean, or replace flow cell 4-8
trace function 3-20–3-22
TRACE key 3-10, 3-20
transient energy 5-21
troubleshooting
contacting Waters 5-20
diagnostic tests 5-1–5-22
hardware 5-21
turning lamp off to conserve lamp life 3-60–3-62
turning lamp on or off
from an external device 3-24
from front panel 3-11
manually 3-60

U

unlock icon 3-6
unpacking the detector 2-5

up/down arrow keys 3-9
user-selected diagnostic tests 5-9–5-19
using
A/B key 3-4
cuvette option 1-4, 1-13, 3-42, 3-56–3-59
diagnostic tests 5-1–5-22
input and output diagnostic tests 5-12
keypad 3-7–3-12
RatioPlot function 3-33
sample and reference energy diagnostic tests 5-11
scale function to zoom 3-20
static flow cell for scanning 3-59
the 2489 as a spectrophotometer 1-12

V

vacuum degassing. See degassing
validation
kits 3-27
procedures 3-27
verifying
detector 3-27–3-29
peak response 3-29
verification algorithm 1-9
viewing events within a method 3-40
voiding the warranty 4-23
voltage offset
function 3-13, 3-16
parameter 3-19

W

warming up the detector 3-28
warning symbols A-2, A-5
warranty
lamp 4-23
voiding 4-23
Waters 600 Series Pump
auto zero connections 2-29
chart mark connections 2-30
configuring the detector lamp signal 2-28
connecting to 2-28–2-31
Inject Start connections 2-31
Waters 717plus Autosampler
auto-zero connections 2-32
connecting to 2-31–2-33
Inject Start connections 2-32
Waters 745/745B/746 Data Module, connecting to 2-23–2-25
Waters data system control 2-13
Waters TaperSlit flow cell
description 1-6–1-8
exploded view 4-13
frontal view 4-11
specifications 4-15, B-5
Waters Technical Service, contacting 2-5, 4-2, 5-20
wavelength
accuracy specifications B-2
calibration 3-11, 3-30
changes, auto-zero on 3-17
changing 3-9, 3-32
ending 3-42
functions in dual 3-31
icon 3-5
parameter 3-18
range specifications B-2
selection C-9–C-11
starting 3-42
timed event parameter 3-35
wrench icon 3-6

Z

zero scan
definition 1-13
parameters 3-45, 3-47

running 3-47
screens 3-49
zoom function 3-20, 3-51